

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 5 :</b> A61K 35/14, 39/00, 37/22 C07K 3/00, 13/00, 15/00 C07K 17/00, C12Q 1/68, 1/00 C12Q 15/00, C12N 1/20, 1/00	<b>A1</b>	<b>(11) International Publication Number:</b> WO 92/20356  <b>(43) International Publication Date:</b> 26 November 1992 (26.11.92)												
<b>(21) International Application Number:</b> PCT/US92/04354 <b>(22) International Filing Date:</b> 22 May 1992 (22.05.92)  <b>(30) Priority data:</b> <table border="0"><tr><td>705,702</td><td>23 May 1991 (23.05.91)</td><td>US</td></tr><tr><td>728,838</td><td>9 July 1991 (09.07.91)</td><td>US</td></tr><tr><td>764,364</td><td>23 September 1991 (23.09.91)</td><td>US</td></tr><tr><td>807,043</td><td>12 December 1991 (12.12.91)</td><td>US</td></tr></table> <b>(71) Applicant (for all designated States except US):</b> LUDWIG INSTITUTE FOR CANCER RESEARCH [US/US]; 1345 Avenue of the Americas, New York, NY 10105 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BOON, Thierry [BE/BE]; VAN DER BRUGGEN, Pierre [BE/BE]; VAN DEN EYNDE, Benoit [BE/BE]; VAN PEL, Aline [BE/BE]; DE PLAEN, Etienne [BE/BE]; LURQUIN, Christophe [BE/BE]; CHOMEZ, Patrick [BE/BE]; Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). TRAVERSA-RI, Catia [IT/IT]; Sesto S. Giovanni, I-20099 Milano (IT).		705,702	23 May 1991 (23.05.91)	US	728,838	9 July 1991 (09.07.91)	US	764,364	23 September 1991 (23.09.91)	US	807,043	12 December 1991 (12.12.91)	US	<b>(74) Agent:</b> HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).  <b>(81) Designated States:</b> AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC (European patent), MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, RU, SD, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US.  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
705,702	23 May 1991 (23.05.91)	US												
728,838	9 July 1991 (09.07.91)	US												
764,364	23 September 1991 (23.09.91)	US												
807,043	12 December 1991 (12.12.91)	US												
<b>(54) Title:</b> TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR REJECTION ANTIGENS AND USES THEREOF  <b>(57) Abstract</b> <p>The invention relates to an isolated DNA sequence which codes for an antigen expressed by tumor cells which is recognized by cytotoxic T cells, leading to lysis of the tumor which expresses it. Also described are cells transfected by the DNA sequence, and various therapeutic and diagnostic uses arising out of the properties of the DNA and the antigen for which it codes.</p>														

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MI	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NI	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

**TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR  
REJECTION ANTIGENS AND USES THEREOF**

This application is a continuation-in-part of Serial Number 807,043, filed December 12, 1991, which is a continuation-in-part of Serial Number 764,364, filed September 23, 1991, which is a continuation-in-part of Serial Number 728,838, filed July 9, 1991, which is a continuation-in-part of Serial Number 705,702, filed May 23, 1991, and now abandoned.

10      **FIELD OF THE INVENTION**

This invention relates in general to the field of immunogenetics as applied to the study of oncology. More specifically, it relates to the study and analysis of mechanisms by which tumors are recognized by the organism's immune system such as through the presentation of so-called tumor rejection antigens, and the expression of what will be referred to herein as "tumor rejection antigen precursors".

**BACKGROUND AND PRIOR ART**

20      The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum<sup>-</sup> antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum<sup>-</sup> antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum<sup>+</sup>" cells). When these tum<sup>+</sup> cells are mutagenized, they are rejected by syngeneic mice, and fail  
10 to form tumors (thus "tum<sup>-</sup>"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum<sup>-</sup> variants fail to form progressive tumors because they elicit an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum<sup>-</sup>" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune  
20 systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum<sup>-</sup> cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory

which permits them to resist subsequent challenge to the same tum<sup>-</sup> variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytotoxic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and



the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and a class of antigens, referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum<sup>-</sup> variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibill, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum<sup>-</sup> antigens are

only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be  $\text{tum}^+$ , such as the line referred to as "P1", and can be provoked to produce  $\text{tum}^-$  variants. Since the  $\text{tum}^-$  phenotype differs from that of the parent cell line, one expects a difference in the DNA of  $\text{tum}^-$  cell lines as compared to their  $\text{tum}^+$  parental lines, and this difference can be exploited to locate the gene of interest in  $\text{tum}^-$  cells. As a result, it was found that genes of  $\text{tum}^-$  variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the  $\text{tum}^-$  antigen are presented by the  $\text{L}^d$  molecule for recognition by CTLs. P91A is presented by  $\text{L}^d$ , P35 by  $\text{D}^d$  and P198 by  $\text{K}^d$ .

It has now been found that the genes which code for the molecules which are processed to form the presentation tumor rejection antigens (referred to as "tumor rejection antigen precursors", "precursor molecules" or "TRAPs" hereafter), are not expressed in most normal adult tissues but are expressed in tumor cells. Genes which code for the TRAPs have now been isolated and cloned, and represent a portion of the invention disclosed herein.

The gene is useful as a source for the isolated and purified tumor rejection antigen precursor and the TRA themselves, either of which can be used as an agent for treating the cancer for which the antigen is a "marker", as well as in various diagnostic and surveillance approaches to oncology, discussed infra. It is known, for example, that tum<sup>-</sup> cells can be used to generate CTLs which lyse cells presenting different tum<sup>-</sup> antigens as well as tum<sup>+</sup> cells. See, e.g., Maryanski et al., Eur. J. Immunol 12: 401 (1982); and Van den Eynde et al., Modern Trends in Leukemia IX (June 1990), the disclosures of which are incorporated by reference. The tumor rejection antigen precursor may be expressed in cells transfected by the gene, and then used to generate an immune response against a tumor of interest.

In the parallel case of human neoplasms, it has been observed that autologous mixed lymphocyte-tumor cell cultures ("MLTC" hereafter) frequently generate responder lymphocytes which lyse autologous tumor cells and do not lyse natural killer targets, autologous EBV-transformed B cells, or autologous fibroblasts (see Anichini et al., Immunol. Today 8: 385-389 (1987)). This response has been particularly well studied for melanomas, and MLTC have been carried out either with peripheral blood cells or with tumor infiltrating lymphocytes. Examples of the literature in this area including Knuth et al., Proc. Natl. Acad. Sci. USA 86: 2804-2802 (1984); Mukherji et al., J. Exp. Med.

158: 240 (1983); Hérin et al., Int. J. Canc. 39: 390-396 (1987); Topalian et al., J. Clin. Oncol 6: 839-853 (1988). Stable cytotoxic T cell clones ("CTLs" hereafter) have been derived from MLTC responder cells, and these clones are specific for the tumor cells. See Mukherji et al., supra, Hérin et al., supra, Knuth et al., supra. The antigens recognized on tumor cells by these autologous CTLs do not appear to represent a cultural artifact, since they are found on fresh tumor cells. Topalian et al., supra; Degiovanni et al., Eur. J. Immunol. 20: 1865-1868 (1990). These observations, coupled with the techniques used herein to isolate the genes for specific murine tumor rejection antigen precursors, have led to the isolation of nucleic acid sequences coding for tumor rejection antigen precursors of TRAs presented on human tumors. It is now possible to isolate the nucleic acid sequences which code for tumor rejection antigen precursors, including, but not being limited to those most characteristic of a particular tumor, with ramifications that are described infra. These isolated nucleic acid sequences for human tumor rejection antigen precursors and applications thereof, as described infra, are also the subject of this invention.

These and various other aspects of the invention are elaborated upon in the disclosure which follows.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 depicts detection of transfectants expressing antigen P815A.

Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

10 Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

Figure 6 shows the results obtained when cells were transfected with the gene from P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment sequence which also express the antigen.

20 Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figur 10 shows the result of Northern blots for MAGE genes on various tissues.

10

Figure 11 presents the data of Figure 13 in table form.

Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

#### BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 is cDNA for part of gene P1A.

SEQ ID NO: 2 presents coding region of cDNA for gene P1A.

10 SEQ ID NO: 3 shows non coding DNA for P1A cDNA which is 3' to the coding region of SEQ ID NO: 2.

SEQ ID NO: 4 is the entire sequence of cDNA for P1A.

SEQ ID NO: 5 is the genomic DNA sequence for P1A.

SEQ ID NO: 6 shows the amino acid sequence for the antigenic peptides for P1A TRA. The sequence is for cells which are A<sup>+</sup> B<sup>+</sup>, i.e., express both the A and B antigens.

SEQ ID NO: 7 is a nucleic acid sequence coding for antigen E.

SEQ ID NO: 8 is a nucleic acid sequence coding for MAGE-1.

20 SEQ ID NO: 9 is the gene for MAGE-2.

SEQ ID NO: 10 is the gene for MAGE-21.

11

SEQ ID NO: 11 is cDNA for MAGE-3.

SEQ ID NO: 12 is the gene for MAGE-31.

SEQ ID NO: 13 is the gene for MAGE-4.

SEQ ID NO: 14 is the gene for MAGE-41.

SEQ ID NO: 15 is cDNA for MAGE-4.

SEQ ID NO: 16 is cDNA for MAGE-5.

SEQ ID NO: 17 is genomic DNA for MAGE-51.

SEQ ID NO: 18 is cDNA for MAGE-6.

SEQ ID NO: 19 is genomic DNA for MAGE-7.

10 SEQ ID NO: 20 is genomic DNA for MAGE-8.

SEQ ID NO: 21 is genomic DNA for MAGE-9.

SEQ ID NO: 22 is genomic DNA for MAGE-10.

SEQ ID NO: 23 is genomic DNA for MAGE-11.

SEQ ID NO: 24 is genomic DNA for smage-I.

SEQ ID NO: 25 is genomic DNA for smage-II.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following

examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

#### Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).



To carry out the selection,  $10^6$  cells of P1.HTR were mixed with  $2-4 \times 10^6$  cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosure of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

10     Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum<sup>r</sup> antigens.

20     Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 µg of cellular DNA and 3 µg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 ul 1M CaCl<sub>2</sub>.

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM  $\text{Na}_2\text{HPO}_4$ , adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells ( $5 \times 10^6$ ) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm<sup>2</sup> tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing  $8 \times 10^6$  cells in 40 ml of medium. In order to estimate the number of transfectants,  $1 \times 10^6$  cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

to be made for the cloning efficiency of P815 cells, known to be about 0.3.

### Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about  $6 \times 10^4$  cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with  $10^6$  irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing  $^{51}\text{Cr}$  labeled P1.HTR target cells ( $2 \times 10^3$  -  $4 \times 10^3$  per well), and chromium release

17

was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

#### Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

10        Prior work had shown that genes coding for tum<sup>r</sup> antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

20        Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately  $9 \times 10^5$

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM MgCl<sub>2</sub>, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of  $2 \times 10^8$  cells/ml ( $OD_{600}=0.8$ ), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

#### Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of  $5 \times 10^6$  PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

#### Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen wer isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278  
10 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of H <sub>2</sub> B <sup>r</sup> transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72



The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

10        Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

20        All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA<sup>+</sup> mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A<sup>+</sup> RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A<sup>+</sup> RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5' end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

10 The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

#### Example 8

20 The Northern analysis described supra suggested that the 5' end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3' primer corresponding to positions 286-266 and a 5' primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

**Example 9**

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

10 The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in  
20 Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

#### Example 10

10 With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions - 0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

20 Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A<sup>-</sup>B<sup>+</sup>", rather than the normal "P1A". The only difference  
10 between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

#### Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

20 The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2<sup>d</sup> haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

10 Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

#### Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2<sup>k</sup>. The cell lines were transfected with genes expressing one of the K<sup>d</sup>, D<sup>d</sup>, and L<sup>d</sup> antigen. Following  
20 transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L<sup>d</sup> is required for presentation of the P1A antigens A and B.



Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No. of clones lysed by the CTL/ no. of H-2B <sup>+</sup> clones*	
	CTL anti-A	CTL anti-B
DAP (H-2 <sup>k</sup> )	0/208	0/194
DAP + K <sup>d</sup>	0/165	0/162
DAP + D <sup>d</sup>	0/157	0/129
DAP + L <sup>d</sup>	25/33	15/20

\*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2<sup>d</sup> class I genes as indicated.

\*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

### Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A<sup>+</sup> B<sup>+</sup> (i.e., characteristic of cells which express both the A and B antigens), and those which are A<sup>-</sup> B<sup>+</sup> were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

#### Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E<sup>-</sup>. This subclone is also HPRT<sup>-</sup>, (i.e., sensitive to HAT medium: 10<sup>-4</sup> M hypoxanthine, 3.8 x 10<sup>-7</sup> aminopterin, 1.6 x 10<sup>-5</sup> M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

#### Example 15

10           The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneo8, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

20           Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 µg) and plasmid DNA (6 µg) were mixed in 940 µl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 µl of 1M CaCl<sub>2</sub> was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

temperature, after which they were applied to 80 cm<sup>2</sup> tissue culture flasks which had been seeded 24 hours previously with  $3 \times 10^6$  MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at  $4 \times 10^6$  cells per 80 cm<sup>2</sup> flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

10      Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200  $\mu$ l of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

20      After 10 days, wells contained approximately  $6 \times 10^4$  cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100  $\mu$ l of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50  $\mu$ l) was harvested and examined

for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is  $6 \times 10^6$  kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interest could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed  $E^+/E^-$  cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ( $4 \times 10^4$ ) had readhered, the CTLs and IL-2 were added thereto. The 50  $\mu$ l of supernatant was removed 24 hours later and transferred to a microplate containing  $3 \times 10^4$  W13 (WEHI-164 clone 13;

Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50  $\mu$ l of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2  $\mu$ g of actinomycin D at 37% in an 8% CO<sub>2</sub> atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF- $\beta$  in RPMI 1640 were added to target cell controls.

10 The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100  $\mu$ l of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were  
20 incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[ 1 - \frac{100 - (\text{OD}_{570} \text{ sample well})}{\text{OD}_{570} \text{ well} + \text{medium}} \right]$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of  $E^+/E^-$  cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

#### Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of  $E^-$  cells ( $4 \times 10^6$  cells/group) were tested following transfection, and  $7 \times 10^4$  independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard  $^{51}\text{Cr}$  release assay, and were found to be lysed as efficiently as the original  $E^+$  cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Exempl 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E<sup>+</sup> contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B<sup>-</sup> and C<sup>-</sup>, just like the recipient cell MEL2.2. It was also found to be HPRT<sup>-</sup>, using standard selection procedures. All E<sup>+</sup> cells used in the work described herein, however, were HPRT<sup>+</sup>.

- 10           It was also possible that an E<sup>+</sup> revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfection with pSVtkneo $\beta$ , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo $\beta$  sequences. Wölfel et al., supra, has shown this to be true. If a normally E<sup>-</sup> cell is transfected with pSVtkneo $\beta$ , then
- 20           sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo $\beta$  sequences. If a normally E<sup>+</sup> cell transfected with pSVtkneo $\beta$  is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin



resistance; however, Southern blot analysis showed loss of several  $\text{neo}^r$  sequences in the variants, showing close linkage between the E gene and  $\text{neo}^r$  gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

#### Example 20

The  $\text{E}^+$  subclone MZ2-MEL 4B was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phase components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so we rescued the transfected sequence by ligating DNA of the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI

fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI/SmaI digested DNA). The band is absent from E<sup>-</sup> antigen loss variants of MZ2-MEL, as seen in Figure 12.

The sequence for the E antigen precursor gene has been determined, and is presented herein:

	1	10	20	30	40	50	60	
3	GGATCCAGGC	CCTGCCAGGA	AAJATAAAG	GGCCCTGCGT	GAGAACAGAG	GGGCTCATTC	60	
61	ACTGCAATGAG	ACTGAGGATG	TCACAGAGTC	CAGCCCAACC	TCTTGTTAGC	ACTGAGAAAG	120	
121	CAGGGCTGTG	CTTGCGGTCT	GCACCCCTGAG	GGCCCGTGGG	TTCCTCTTCC	TGGAGCTCCA	180	
181	GGAAACAGGC	AGTGAGGCGT	TGCTCTGAGA	CAGTATCCCT	AGCTCACAGA	GCAGAGGATG	240	
241	CACAGGCTGT	GCCAGCACTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300	
301	CAGGACACAT	AGGATCTCAC	AGAGTCTGAC	CTCACCCTCC	TACTGTCACT	CCTGTAGAA	360	
361	CGACCTCTGC	TGGCCCGGCTG	TACCCCTGAGT	ACCCCTCTCAC	TTCCTCCTTC	AGGTTTTTCA	420	
421	GGGACAGGCC	AACCCAGAGG	ACAGGATTCG	CTGGAGGCCA	CAGAGGAGCA	CCAAAGAGAA	480	
481	GATCTGTAAAG	TAGGCTCTTG	TTAGAGTCTC	CAGGCTTCAG	TTCCTCAGCTG	AGGCTCTCTA	540	
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGGT	CTTCATTGCG	CAGCTCCTGC	CCCACTCCCT	600	
601	GCCTGCTGCC	CTGAGGAGAG	TCAATCATGT	TCTTGAGGAG	AGGAGTCTGC	ACTGCAAGCC	660	
661	TGAGGAAAGCC	CTTGAGGCCC	AACAAGAGGC	CTTGCCCTGG	TGTGTGTGCA	GGCTGCCACC	720	
721	TCTCTCTCTT	CTCTCTGTGT	CCTGGGCAAC	CTGGAGGAGG	TGCCCACTGC	TGGGTCAACA	780	
781	GATCTCTCCC	AGAGTCTCTA	GGAGGCTCTC	GCCTTTCCTA	CTACCATCAA	CTTCACTCGA	840	
841	CAGAGGCAAC	CCAGTGAAGG	TTCCAGCAGC	CGTGAAGAGG	AGGGATCAAG	CACCTCTTGT	900	
901	ATCTGTGAGT	CCTTGTCTCC	AGCACTAATC	ACTAAGAAAG	TGGTGTATTT	GGTTGGTTTT	960	
961	CTGCTCCCTA	AATATCGAGC	CAGGGAAGCA	GTCACAAAAG	CAGAAATGCT	GGAGAGTGTG	1020	
1021	ATCAAAATTT	ACAAGCACTG	TTTTCTGTAG	ATCTTCCGCA	AAGCCCTCTA	GTCCCTTGCA	1080	
1081	CTGTCTCTTG	GCAATTGAGT	GAAAGAAAGCA	GACCCCAACC	GCCACTCTTA	TGTCTCTTGT	1140	
1141	ACCTGCTCTG	GTCTCTCTTA	TGATGGGCTG	CTGGGTGATA	ATCAGATCAT	GCCCAAGACA	1200	
1201	GCTTCTCTGA	TAAATTGTCT	GCTCATGATT	GCAATGGAGG	GCGGCCATGC	TCTTGAGGAG	1260	
1261	GAAATCTTGG	AGGAGCTGAG	TGTGTGTGAG	TTGTATGATG	GAAGGAGGCA	CAGTGCCTAT	1320	
1321	GGGAGGCCCC	GGAGGCTGCT	CACCCAAAGT	TTGGTCCAGG	AAAGGTACCT	GGAGTACGGC	1380	
1381	AGGTGCTCGA	CAGTGAATCC	GCACGCTATG	ASTTCTCTGT	GGGTGCAAGG	GGCCTCTCTG	1440	
1441	AAACCAGCTA	TGTGAAGTGC	CTTGAGTATG	TGATCAAGGT	CAGTGCAAGA	GTTCGCTTTT	1500	
1501	TCTTCCCATC	CCTGCTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1560	
1561	TTGCAGCCAA	GGCCAGTGGG	AGGGGGAATG	GGCCATGTGA	CCTTCCAGGG	CCGCTCTCCG	1620	
1621	CAGCTTCCCC	TGCTCTGTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGGGTCTAGT	1680	
1681	GTTCCTAGTA	GTAGGTTTCT	GTTCATTTGG	GTGACTTGGG	GATTTATCTT	TGTTCTCTTT	1740	
1741	TGGAAATGTT	CAATGTCTTT	TTTTTAAAGG	ATGTTTGAAT	GAACTTCAGC	ATCCAAAGTT	1800	
1801	ATGAATGACA	GCACTCACAC	AGTTCTGTGT	ATATAGTTTA	AGGTTAAGAG	TCTTGTGTTT	1860	
1861	TATTCAGATT	GGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAAATAACAG	AGTGGAAATA	1920	
1921	GTACTTAGAA	ATGTGAATAA	TGAGCACTAA	ATAGATGAGG	ATAAAGAACT	AAAGAAATTA	1980	
1981	AGAGATAGTC	AATTCCTGCC	TTATACCTCA	GTCTATTTCT	TAAATTTTCT	AAAGATATAT	2040	
2041	GCATACCTGG	ATTTCTCTGG	CTTCTTTGAG	AATGTAAAGG	AATTTAAATC	TGAATAAAGA	2100	
2101	ATCTCTCTGT	TTCACTGGCT	CTTTCTTCTT	CCATGCACTG	AGCATCTGCT	TTTTGGAAAG	2160	
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCGACTTC	ATACCCACCC	ATAGGTTCTG	2220	
2221	AGATCTTAGG	AGCTGCACTC	ACCTAATCGA	GGTGGCAAGA	TGTCCTCTAA	AGATGTAGGG	2280	
2281	AAAGTGTAGA	GAGGGGTGAG	GGTGTGGGGC	TCCGGGTGAG	AGTGTGTAG	TGTCAATGCC	2340	
2341	CTGAGCTGGG	GCATTTTGGG	CTTTGGGAAA	CTGCAATTCC	TTCTGGGGGA	GTGATTGTA	2400	
2401	ATGATCTTGG	GTGATCC					2418	

Exempl 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E<sup>+</sup>" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5' end of the cDNA. The three exons comprise 65, 73, and 1551 base pairs. An ATG is located at position 66 of exon 3, followed by an 828 base pair reading frame.

Example 22

To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E<sup>-</sup> cells. Figure 8 shows the boundaries of the three segments.

Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

### Example 23

The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, "mage -1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them. These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments which follow indicate, however, the members of the MAGE family are not at all restricted to melanoma tumors;

rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

#### Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the mage-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2.

Amplification by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with the DNA of the E<sup>-</sup> variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E<sup>+</sup> melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Exempl 25

In order to evaluate the expression of gene mage-1 by various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals (Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these culture cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2 proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes mage-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligo- nucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers

corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than 1/300<sup>th</sup> that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also expressed all three genes whereas others expressed only mage-2 and 3 or only mage-3. It is impossible to exclude formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but that of yet another closely related gene that would share the sequence of the priming and hybridizing oligonucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this point.



Example 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneo $\beta$ . Three of them yielded neo<sup>r</sup> transfectants that

10 stimulated TNF release by anti-E CTL clone 82/30, which is CD8<sup>+</sup> (Figure 10). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL also showed high sensitivity to lysis by these anti-E CTL. These two melanomas were those that expressed

20 mage-1 gene (Figure 13). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes

mage 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

#### Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the  
10 nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E<sup>-</sup> cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation  
20 of antigen -E precursor DNA, the F<sup>-</sup> variant was transfected with genomic DNA from F<sup>+</sup> cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-

F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

#### Example 28

Following identification of F<sup>+</sup> cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F<sup>+</sup> cell line M22-MEL.43 was prepared, again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA  
10 from each group was transfected into M22-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 genitacin resistant transfectants.

#### Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the  
20 expression of antigen M22-E, was labelled with 32p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone M22-MEL2.2. Hybridization conditions included 50  $\mu$ l/cm<sup>2</sup> of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where th 2.4 kb probes had been labelled with [ $\alpha^{32}$ p]dCTP (2-3000

Ci/mole), at  $3 \times 10^6$  cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

10 In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

#### Example 30

20 The cDNA coding for mage 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express mage 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for mage 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as mage 4.

#### Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which

showed homology to mage 1 but not mage 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "mage 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

### Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA  
10 from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT), and CHO10: (GAAGAGGAGGGGCCAAG). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1  $\mu$ g of RNA was diluted to a total volume of 20  $\mu$ l, using 2  $\mu$ l of 10x PCR buffer, 2  $\mu$ l of each of 10 mM dNTP, 1.2  $\mu$ l of 25 mM  $MgCl_2$ , 1  $\mu$ l of an 80 mM solution of CHO9, described supra, 20 units of RNasin, and 200 units of M-MLV reverse transcriptase. This was followed by  
20 incubation for 40 minutes at 42°C. PCR amplification followed, using 8  $\mu$ l of 10x PCR buffer, 4.8  $\mu$ l of 25 mM  $MgCl_2$ , 1  $\mu$ l of CHO10, 2.5 units of *Thermus aquaticus* ("Taq") polymerase, and water to a total volume of 100  $\mu$ l. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten  $\mu$ l of each reaction were then size fractionated on agarose gel,

followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CHO18 (TCTTGATACCTGGAGTCC). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from mage 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to mage 4 and 5. These results indicate a sequence differing from previously identified mage 1, 2, 3, 4 and 5, and is named mage 6.

### Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb mage 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for mage 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from mages 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded mage 8-11.

### Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of mage 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether

synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr was shown to be best. The assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

#### Example 35

Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

#### Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for

pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

The foregoing disclosure, including the examples, places many tools of extreme value in the hands of the skilled artisan. To begin, the examples identify and provide a methodology for isolating nucleic acid molecules which code for tumor rejection antigen precursors as well as the nucleic acid molecules complementary thereto. It is  
10 known that DNA exists in double stranded form, and that each of the two strands is complementary to the other. Nucleic acid hybridization technology has developed to the point where, given a strand of DNA, the skilled artisan can isolate its complement, or synthesize it.

"Nucleic acid molecule" as used herein refers to all species of DNA and RNA which possess the properties discussed supra. Genomic and complementary DNA, or "cDNA" both code for particular proteins, and as the examples directed to isolation of MAGE coding sequences show, this  
20 disclosure teaches the artisan how to secure both of these.

Similarly, RNA molecules, such as mRNA can be secured. Again, with reference to the skilled artisan, once one has a coding sequence in hand, mRNA can be isolated or synthesized.

Complementary sequences which do not code for TRAP, such as "antisense DNA" or mRNA are useful, e.g., in



probing for the coding sequence as well as in methodologies for blocking its expression.

It will also be clear that the examples show the manufacture of biologically pure cultures of cell lines which have been transfected with nucleic acid sequences which code for or express the TRAP molecules. Such cultures can be used as a source for tumor rejection antigens, e.g., or as therapeutics. This aspect of the invention is discussed infra.

10        Cells transfected with the TRAP coding sequences may also be transfected with other coding sequences. Examples of other coding sequences include cytokine genes, such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene transfection is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of the cells in vivo. The art is well aware of therapies where interleukin transfectants have been administered to  
20        subjects for treating cancerous conditions. In a particularly preferred embodiment, cells are transfected with sequences coding for each of (i) a TRAP molecule, (ii) an HLA/MHC molecule, and (iii) a cytokine.

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA,

additional transfection may not be necessary although further transformation could be used to cause over-expression of the antigen. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that transfection with one additional sequence does not preclude further transfection with other sequences.

10       The term "biologically pure" as used in connection with the cell line described herein simply means that these are essentially free of other cells. Strictly speaking, a "cell line" by definition is "biologically pure", but the recitation will establish this fully.

20       Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for the TRAP of interest is operably linked to a promoter. The promoter may be a strong promoter, such as those well known to the art, or a differential promoter, i.e., one which is operative only in specific cell types. The expression vectors may also contain all or a part of a viral or bacterial genome, such as vaccinia virus or BCG. Such vectors are especially useful in preparing vaccines.

      The expression vectors may incorporate several coding sequences, as long as the TRAP sequence is contained therein. The cytokine and/or MHC/HLA genes discussed supra may be included in a single vector with the TRAP sequence. Where this is not desired, then an expression system may be

provided, where two or more separate vectors are used where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-transfection is a well known technique, and the artisan in this field is expected to have this technology available for utilization. The vectors may be constructed so that they code for the TRA molecule directly, rather than the TRAP molecule. This eliminates the need for post-translational processing.

10           As the foregoing discussion makes clear, the sequences code for "tumor rejection antigen precursors" ("TRAPs") which, in turn, are processed into tumor rejection antigens ("TRAs"). Isolated forms of both of these categories are described herein, including specific examples of each. Perhaps their most noteworthy aspect is as vaccines for treating various cancerous conditions. The evidence points to presentation of TRAs on tumor cells, followed by the development of an immune response and deletion of the cells. The examples show that when various TRAs are  
20 administered to cells, a CTL response is mounted and presenting cells are deleted. This is behavior characteristic of vaccines, and hence TRAPs, which are processed into TRAs, and the TRAs themselves may be used, either alone or in pharmaceutically appropriate compositions, as vaccines. Similarly, presenting cells may be used in the same manner, either alone or as combined with ingredients to yield pharmaceutical compositions. Additional materials which may be used as vaccines include

isolated cells which present the TRA molecule on their surface, as well as TRAP fragments, mutated viruses, especially etiolated forms, and transfected bacteria. "Fragments" as used herein refers to peptides which are smaller than the TRA, but which possess the properties required of a vaccine, as discussed supra. Another vaccine comprises or consists of complexes of TRA and HLA molecule. Vaccines of the type described herein may be used preventively, i.e., via administration to a subject in an amount sufficient to prevent onset of a cancerous condition.

The generation of an immune response, be it T-cell or B-cell related, is characteristic of the effect of the presented tumor rejection antigen. With respect to the B-cell response, this involves, inter alia, the generation of antibodies to the TRA, i.e., which specifically bind thereto. In addition, the TRAP molecules are of sufficient size to render them immunogenic, and antibodies which specifically bind thereto are a part of this invention.

These antibodies may be polyclonal or monoclonal, the latter being prepared by any of the well recognized methodologies for their preparation which need not be repeated here. For example, mAbs may be prepared using an animal model, e.g., a Balb/C mouse or in a test tube, using, e.g., EBV transformants. In addition, antiserum may be isolated from a subject afflicted with a cancerous condition where certain cells present a TRA. Such

antibodies may also be generated to epitopes defined by the interaction of TRA and HLA/MHC molecules.

Review of the foregoing disclosure will show that there are a number of facets to the system which may be referred to as "tumor rejection antigen presentation and recognition". Recognition of these phenomena has diagnostic consequences. For example, the existence of specific CTL clones, or antibodies to the TRA makes it possible to diagnose or monitor cancerous conditions (explained infra), by monitoring the CTLs in a sample from a subject, binding of antibodies to TRAs, or the activity of anti-TRA CTLs in connection with subject samples. Similarly, the expression of nucleic acid molecules for TRAs can be monitored via amplification (e.g., "polymerase chain reaction"), anti-sense hybridization, probe technologies, and so forth. Various subject samples, including body fluids (blood, serum, and other exudates, e.g.), tissues and tumors may be so assayed.

A particular manner of diagnosis is to use an adaptation of the standard "tuberculin test" currently used for diagnosis of tuberculosis. This standard skin test administers a stable form of "purified protein derivative" or "PPD" as a diagnostic aid. In a parallel fashion, TRAs in accordance with this invention may be used in such a skin test as a diagnostic aid or monitoring method.

The term "cancerous condition" is used herein to embrace all physiological events that commence with the initiation of the cancer and result in final clinical

manifestation. Tumors do not spring up "ab initio" as visible tumors; rather there are various events associated with the transformation of a normal cell to malignancy, followed by development of a growth of biomass, such as a tumor, metastasis, etc. In addition, remission may be conceived of as part of "a cancerous condition" as tumors seldom spontaneously disappear. The diagnostic aspects of this invention include all events involved in carcinogenesis, from the first transformation to malignancy of a single cell, through tumor development and metastasis, as well as remission. All are embraced herein.

Where "subject" is used, the term embraces any species which can be afflicted with a cancerous condition. This includes humans and non-humans, such as domesticated animals, breeding stock, and so forth.

There are therapeutic aspects of this invention as well. The efficacy of administration of effective amounts of TRAPs and TRAs as vaccines has already been discussed supra. Similarly, one may develop the specific CTLs in vitro and then administer these to the subject. Antibodies may be administered, either polyclonal or monoclonal, which specifically bind to cells presenting the TRA of interest. These antibodies may be coupled to specific antitumor agents, including, but not being limited to, methotrexate radio-iodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Thus, "targeted" antibody therapy is included herein, as is the

application of deletion of the cancerous cells by the use of CTLs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

## (1) GENERAL INFORMATION:

- (i) APPLICANTS: Boon, Thierry, Van den Eynde, Benoît
- (ii) TITLE OF INVENTION: Isolated And Purified DNA Sequenc  
Coding Antigen Expressed By Tumor Cells And Recognized By  
Cytotoxic T Cells, And Uses Thereof
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Felfe & Lynch
  - (B) STREET: 805 Third Avenue
  - (C) CITY: New York City
  - (D) STATE: New York
  - (F) ZIP: 10022
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
  - (B) COMPUTER: IBM
  - (C) OPERATING SYSTEM: PC-DOS
  - (D) SOFTWARE: Wordperfect
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 07/807,043
  - (B) FILING DATE: 12-DECEMBER-1991
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 07/764,364
  - (B) FILING DATE: 23-SEPTEMBER-1991
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 07/728,838
  - (b) FILING DATE: 9-JULY-1991
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 07/705,702
  - (B) FILING DATE: 23-May-1991
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Hanson, Norman D.
  - (B) REGISTRATION NUMBER: 30,946
  - (C) REFERENCE/DOCKET NUMBER: LUD 253.4
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (212) 688-9200
  - (B) TELEFAX: (212) 838-3884

**SUBSTITUTE SHEET**



## (2) INFORMATION FOR SEQUENCE ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 462 bas pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACCACAGGAG	AATGAAAAGA	ACCCGGGACT	CCCAAAGACG	CTAGATGTGT	50
GAAGATCCTG	ATCACTCATT	GGGTGTCTGA	GTTCTGCGAT	ATTCATCCCT	100
CAGCCAATGA	GCTTACTGTT	CTCGTGGGGG	GTTTGTGAGC	CTTGGGTAGG	150
AAGTTTTGCA	AGTTCCGCCT	ACAGCTCTAG	CTTGTGAATT	TGTACCCTTT	200
CACGTAAAAA	AGTAGTCCAG	AGTTTACTAC	ACCCTCCCTC	CCCCCTCCCA	250
CCTCGTGCTG	TGCTGAGTTT	AGAAGTCTTC	CTTATAGAAG	TCTTCCGTAT	300
AGAACTCTTC	CGGAGGAAGG	AGGGAGGACC	CCCCCCTTTT	GCTCTCCCAG	350
CATGCATTGT	GTCAACGCCA	TTGCACTGAG	CTGGTCGAAG	AAGTAAGCCG	400
CTAGCTTGCG	ACTCTACTCT	TATCTTAACT	TAGCTCGGCT	TCCTGCTGGT	450
ACCCTTTGTG	CC				462

SUBSTITUTE SHEET

## (2) INFORMATION FOR SEQUENCE ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 675 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT	48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly	
5 10 15	
GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA	96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu	
20 25 30	
GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA	144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr	
35 40 45	
AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG	192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln	
50 55 60	
TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC	240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser	
65 70 75 80	
TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC	288
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr	
85 90 95	
GAC GAC GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT	336
Asp Asp Glu Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp	
100 105 110	
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG	384
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu	
115 120 125	
GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG	432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met	
130 135 140	
GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT GGC CAT CAT TTA AGG AAG	480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys	
145 150 155 160	
AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC	528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe	
165 170 175	

63

CTG	GTG	TCT	ATA	CCA	GTG	AAC	CCT	AAG	GAA	CAA	ATG	GAG	TGT	AGG	TGT	576
Leu	Val	Ser	Ile	Pro	Val	Asn	Pro	Lys	Glu	Gln	Met	Glu	Cys	Arg	Cys	
			180					185					190			
GAA	AAT	GCT	GAT	GAA	GAG	GTT	GCA	ATG	GAA	GAG	GAA	GAA	GAA	GAA	GAG	624
Glu	Asn	Ala	Asp	Glu	Glu	Val	Ala	Met	Glu	Glu	Glu	Glu	Glu	Glu	Glu	
		195					200				210					
GAG	GAG	GAG	GAG	GAA	GAG	GAA	ATG	GGA	AAC	CCG	GAT	GGC	TTC	TCA	CCT	672
Glu	Glu	Glu	Glu	Glu	Glu	Glu	Met	Gly	Asn	Pro	Asp	Gly	Phe	Ser	Pro	
220						225				230					235	
TAG																675

SUBSTITUTE SHEET

- (2) INFORMATION FOR SEQUENCE ID NO: 3:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 228 base pairs  
    (B) TYPE: nucleic acid  
    (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: genomic DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG TTGTTTTTTT    60
TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA ATTTGATTTT GTTCTAAAGT    120
TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACTTT CATATGATAC    180
ATAGGATTAC ACTTGACCT GTTAAAAATA AAAGTTTGAC TTGCATAC                    228
```

**SUBSTITUTE SHEET**

- (2) INFORMATION FOR SEQUENCE ID NO: 4:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1365 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```

ACCACAGGAG AATGAAAAGA ACCCGGGGACT CCCAAAGACG CTAGATGTGT      50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT      100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG      150
AAGTTTGTCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT      200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA      250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT      300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCTTT GCTCTCCCAG      350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG      400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT      450
ACCCTTTGTG CC                                     462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA      504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG      546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC      588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC      630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC      672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG      714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC      756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA      840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA      882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT      924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT      966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG     1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT     1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAG GAG GAG     1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT     1134
TAG                                                         1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG     1187
TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTGTAGT AATCCAGAAA     1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT     1287
CTGACTGCAT GGTGAACCTT CATATGATAC ATAGGATTAC ACTTGACCT      1337
GTAAAAATA AAAGTTTGAC TTGCATAC                               1365

```

**SUBSTITUTE SHEET**

- (2) INFORMATION FOR SEQUENCE ID NO: 5:  
 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 4698 base pairs  
     (B) TYPE: nucleic acid  
     (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: genomic DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```

ACCACAGGAG AATGAAAAGA ACCCGGGGACT CCCAAAGACG CTAGATGTGT      50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT      100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTTAGG      150
AAGTTTGTGA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT      200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA      250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT      300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCTTT GCTCTCCAG      350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG      400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT      450
ACCCTTTGTG CC                                     462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA      504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG      546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC      588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC      630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC      672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG      714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC      756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA      840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA      882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC T          916
GTGAGTAACC CGTGGTCTTT ACTCTAGATT CAGGTGGGGT GCATTCTTTA      966
CTCTTGCCCA CATCTGTAGT AAAGACCACA TTTTGGTTGG GGGTCATTGC     1016
TGGAGCCATT CCTGGCTCTC CTGTCCACGC CTATCCCCGC TCCTCCCATC     1066
CCCCACTCCT TGCTCCGCTC TCTTTCCTTT TCCCACCTTG CCTCTGGAGC     1116
TTCAGTCCAT CCTGCTCTGC TCCCTTTCCC CTTTGCTCTC CTTGCTCCCC     1166
TCCCCCTCGG CTCAACTTTT CGTGCCCTCT GCTCTCTGAT CCCCACCCTC     1216
TTCAGGCTTC CCCATTGTCT CCTCTCCCGA AACCCCTCCC TTCCTGTTC     1266
CCTTTTCGCG CCTTTTCTTT CCTGCTCCCC TCCCCCTCCC TATTTACCTT     1316
TCACCAGCTT TGCTCTCCCT GCTCCCTCC CCCTTTTGCA CCTTTTCTTT     1366
TCCTGCTCCC CTCCCCCTCC CCTCCCTGTT TACCCTTCAC CGCTTTTCTT     1416
CTACCTGCTT CCCTCCCCCT TGCTGCTCCC TCCCTATTG CATTTCGGG      1466
TGCTCCTCCC TCCCCCTCCC CCTCCCTCCC TATTGTCATT TTCGGGTGCT     1516
CCTCCCTCCC CCTCCCCAGG CCTTTTTTTT TTTTTTTTTT TTTTTTTTTT     1566
TTGGTTTTTC GAGACAGGGT TTCTCTTGT ATCCCTGGCT GTCCTGGCAC     1616
TCACTCTGTA GACCAGGCTG GCCTCAAAC CAGAAATCTG CCTGCCTCTG     1666
CCTCCCAAAT GCTGGGATTA AAGGCTTGCA CCAGGACTGC CCCAGTGCAG     1716
GCCTTTCCTT TTTCTCCTCT CTGGTCTCCC TAATCCCTTT TCTGCATGTT     1766
AACTCCCTTT TTGGCACCTT TCCTTTACAG GACCCCTCC CCCTCCCTGT     1816
TTCCCTTCCG GCACCCTTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC     1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT     1916
GCCCGTTCC CCTTTTTTGT GCCTTTCCTC CTGGCTCCCC TCCACCTTCC     1966
AGCTCACCTT TTTGTTTGTG TGGTTGTTG GTTGTGTTGG TTGCTTTTTT     2016
TTTTTTTTTT GCACCTTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC     2066
CCTCTGTGTG CCTTTCCTGT TCCCTCCCC TCGCTGGCTC CCCCTCCCTT     2116

```

SUBSTITUTE SHEET

TCTGCCTTTC	CTGTCCCTGC	TCCCTTCTCT	GCTAACCTTT	TAATGCCTTT	2166
CTTTTCTAGA	CTCCCCCTC	CAGGCTTGCT	GTTTGCTTCT	GTGCACTTTT	2216
CCTGACCCTG	CTCCCCCTCC	CCTCCCAGCT	CCCCCTCTT	TTCCCACCTC	2266
CCTTTCTCCA	GCCTGTCACC	CCTCCTTCTC	TCCTCTCTGT	TTCTCCCCTC	2316
TCCTGCTTCC	TTTACCCCTT	CCCTCTCCCT	ACTCTCCTCC	CTGCCTGCTG	2366
GACTTCCTCT	CCAGCCGCCC	AGTTCCCTGC	AGTCCTGGAG	TCTTTCCTGC	2416
CTCTCTGTCC	ATCACTTCCC	CCTAGTTTCA	CTTCCCTTTC	ACTCTCCCCCT	2466
ATGTGTCTCT	CTTCCCTATCT	ATCCCTTCCCT	TTCTGTCCCC	TCTCCTCTGT	2516
CCATCACCTC	TCTCCTCCCT	TCCCTTTCCCT	CTCTCTTCCA	TTTTCTTCCA	2566
CCTGCTTCTT	TACCCTGCCT	CTCCCATTCG	CCTCTTACCT	TTATGCCCCAT	2616
TCCATGTCCC	CTCTCAATTG	CCTGTCCCCT	TGTGCTCCCT	CACATCTTCC	2666
ATTTCCCTCT	TTCTCCCTTA	GCCTCTTCTT	CCTCTTCTCT	TGTATCTCCC	2716
TTCCCTTTGC	TTCTCCCTCC	TCCTTTCCCC	TTCCCTTATG	CCCTCTACTC	2766
TACTTGATCT	TCTCTCCTCT	CCACATACCC	TTTTTCCCTT	CCACCCTGCC	2816
CTTTGTCCCC	AGACCCTACA	GTATCCTGTG	CACAGGAAGT	GGGAGGTGCC	2866
ATCAACAACA	AGGAGGCAAG	AAACAGAGCA	AAATCCCAAA	ATCAGCAGGA	2916
AAGGCTGGAT	GAAAATAAGG	CCAGGTTCTG	AGGACAGCTG	GAATCTAGCC	2966
AAGTGGCTCC	TATAACCCTA	AGTACCAAGG	GAGAAAGTGA	TGGTGAAGTT	3016
CTTGATCCTT	GCTGCTTCTT	TTACATATGT	TGGCACATCT	TTCTCAAATG	3066
CAGGCCATGC	TCCATGCTTG	GCGCTTGCTC	AGCGTGGTTA	AGTAATGGGA	3116
GAATCTGAAA	ACTAGGGGCC	AGTGGTTTGT	TTTGGGGACA	AATTAGCACG	3166
TAGTGATATT	TCCCCCTAAA	AATTATAACA	AACAGATTCA	TGATTTGAGA	3216
TCCTTCTACA	GGTGAGAAGT	GGAAAAATTG	TCACTATGAA	GTTCTTTTTTA	3266
GGCTAAAGAT	ACTTGGAACC	ATAGAAGCGT	TGTTAAAATA	CTGCTTTCTT	3316
TTGCTAAAT	ATTCTTTCTC	ACATATTCAT	ATTCTCCAG		3355
GT GTT CCT	GGC CAT CAT	TTA AGG AAG	AAT GAA GTG	AAG TGT	3396
AGG ATG ATT	TAT TTC TTC	CAC GAC CCT	AAT TTC CTG	GTG TCT	3438
ATA CCA GTG	AAC CCT AAG	GAA CAA ATG	GAG TGT AGG	TGT GAA	3480
AAT GCT GAT	GAA GAG GTT	GCA ATG GAA	GAG GAA GAA	GAA GAA	3522
GAG GAG GAG	GAG GAG GAA	GAG GAA ATG	GGA AAC CCG	GAT GGC	3564
TTC TCA CCT	TAG				3576
GCATGCAGGT	ACTGGCTTCA	CTAACCAACC	ATTCCTAACA	TATGCCTGTA	3626
GCTAAGAGCA	TCTTTTAAAA	AAATATTATT	GGTAAACTAA	ACAATTGTTA	3676
TCTTTTACCA	TTAATAAGTA	TTAAATTAAT	CCAGTATACA	GTTTAAAGAA	3726
CCCTAAGTTA	AACAGAAGTC	AATGATGTCT	AGATGCCTGT	TCTTTAGATT	3776
GTAGTGAGAC	TACTTACTAC	AGATGAGAAG	TTGTTAGACT	CGGGAGTAGA	3826
GACCAGTAAA	AGATCATGCA	GTGAAATGTG	GCCATGGAAA	TCCCATATTG	3876
TTCTTATAGT	ACCTTTGAGA	CAGCTGATAA	CAGCTGACAA	AAATAAGTGT	3926
TTCAAGAAAG	ATCACACGCC	ATGGTTCACA	TGCAAATTAT	TATTTTGTCTG	3976
TTCTGATTTT	TTTCATTTCT	AGACCTGTGG	TTTTAAAGAG	ATGAAAATCT	4026
CTTAAATTTT	CCTTCATCTT	TAATTTTCCT	TAACCTTAGT	TTTTTCTACT	4076
TAGAATTCAA	TTCAAATTCT	TAATTCAATC	TTAATTTTAA	GATTTCTTAA	4126
AATGTTTTTT	AAAAAAAATG	CAAATCTCAT	TTTTAAGAGA	TGAAAGCAGA	4176
GTAAGTGGG	GGCTTAGGGA	ATCTGTAGGG	TTGCGGTATA	GCAATAGGGA	4226
GTTCTGGTCT	CTGAGAAGCA	GTCAGAGAGA	ATGGAAAACC	AGGCCCTTGC	4276
CAGTAGGTTA	GTGAGGTGTA	TATGATCAGA	TTATGGACAC	TCTCCAAATC	4326
ATAAATACTC	TAACAGCTAA	GGATCTCTGA	GGGAAACACA	ACAGGGGAAT	4376
ATTTTAGTTT	CTCCTTGAGA	AACAATGACA	AGACATAAAA	TTGGCAAGAA	4426
AGTCAGGAGT	GTATTCTAAT	AAGTGTGCT	TATCTCTTAT	TTTCTTCTAC	4476
AGTTGCCAAG	CCCAGAAGAA	AGAAATGGAC	AGCGGAAGAA	GTGGTTGTTT	4526
TTTTTTCCCC	TTCATTAATT	TTCTAGTTTT	TAGTAATCCA	GAAAATTTGA	4576
TTTTGTTCTA	AAGTTTATTA	TGCAAAGATG	TCACCAACAG	ACTTCTGACT	4626
GCATGGTGAA	CTTTCATATG	ATACATAGGA	TTACACTTGT	ACCTGTTAAA	4676
AATAAAAGTT	TGACTTGCAAT	AC			4698

## SUBSTITUTE SHEET

- (2) INFORMATION FOR SEQUENCE ID NO: 6:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: protein
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe  
5



69

- (2) INFORMATION FOR SEQUENCE ID NO: 7:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2418 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```

GGATCCAGGC CCTGCCAGGA AAAATATAAG GGCCCTGCGT GAGAACAGAG      50
GGGGTCATCC ACTGCATGAG AGTGGGGATG TCACAGAGTC CAGCCCACCC      100
TCCTGGTAGC ACTGAGAAGC CAGGGCTGTG CTTGCGGTCT GCACCCTGAG      150
GGCCCGTGGA TTCCTCTTCC TGGAGCTCCA GGAACCAGGC AGTGAGGCCT      200
TGGTCTGAGA CAGTATCCTC AGGTCACAGA GCAGAGGATG CACAGGGTGT      250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA      300
CAGGACACAT AGGACTCCAC AGAGTCTGGC CTCACCTCCC TACTGTCAGT      350
CCTGTAGAAT CGACCTCTGC TGGCCGGCTG TACCCTGAGT ACCCTCTCAC      400
TTCCTCCTTC AGGTTTTCAG GGGACAGGCC AACCAGAGG ACAGGATTCC      450
CTGGAGGCCA CAGAGGAGCA CCAAGGAGAA GATCTGTAAG TAGGCCTTTG      500
TTAGAGTCTC CAAGGTTTCAG TTCTCAGCTG AGGCCTCTCA CACACTCCCT      550
CTCTCCCCAG GCCTGTGGGT CTTTATTGCC CAGCTCCTGC CCACACTCCT      600
GCCTGCTGCC CTGACGAGAG TCATCATGTC TCTTGAGCAG AGGAGTCTGC      650
ACTGCAAGCC TGAGGAAGCC CTTGAGGCCA AACAAGAGGC CCTGGGCCTG      700
GTGTGTGTGC AGGCTGCCAC CTCCTCCTCC TCTCCTCTGG TCCTGGGCAC      750
CCTGGAGGAG GTGCCCCTG CTGGGTCAAC AGATCCTCCC CAGAGTCCTC      800
AGGGAGCCTC CGCCTTTCCC ACTACCATCA ACTTCACTCG ACAGAGGCAA      850
CCCAGTGAGG GTTCCAGCAG CCGTGAAGAG GAGGGGCCAA GCACCTCTTG      900
TATCCTGGAG TCCTTGTTCC GAGCAGTAAT CACTAAGAAG GTGGCTGATT      950
TGGTTGGTTT TCTGCTCCTC AAATATCGAG CCAGGGAGCC AGTCACAAAG     1000
GCAGAAATGC TGGAGAGTGT CATCAAAAAT TACAAGCACT GTTTTCTCTGA     1050
GATCTTCGGC AAAGCCTCTG AGTCCTTGCA CTGGTCTTTT GGCATTGACG     1100
TGAAGGAAGC AGACCCCAAC GGCCACTCCT ATGTCCTTGT CACCTGCCTA     1150
GGTCTCTCCT ATGATGGCCT GCTGGGTGAT AATCAGATCA TGCCCAAGAC     1200
AGGCTTCCTG ATAATTGTCC TGGTCATGAT TGCAATGGAG GCGCGCCATG     1250
CTCCTGAGGA GGAAATCTGG GAGGAGCTGA GTGTGATGGA GGTGTATGAT     1300
GGGAGGGAGC ACAGTGCCTA TGGGGAGCCC AGGAAGCTGC TCACCCAAGA     1350
TTTGGTGCAG GAAAAGTACC TGGAGTACGG CAGGTGCCGG ACAGTGATCC     1400
CGCACGCTAT GAGTTCCTGT GGGGTCCAAG GGCCCTCGCT GAAACCAGCT     1450
ATGTGAAAGT CCTTGAGTAT GTGATCAAGG TCAGTGCAAG AGTTCGCTTT     1500
TTCTTCCCAT CCCTGCGTGA AGCAGCTTTG AGAGAGGAGG AAGAGGGAGT     1550
CTGAGCATGA GTTGACAGCA AGGCCAGTGG GAGGGGGACT GGGCCAGTGC     1600
ACCTTCCAGG GCCGCGTCCA GCAGCTTCCC CTGCCTCGTG TGACATGAGG     1650
CCCATTCTTC ACTCTGAAGA GAGCGGTCAG TGTTCTCAGT AGTAGGTTTC     1700
TGTTCTATTG GGTGACTTGG AGATTTATCT TTGTTCTCTT TTGGAATTGT     1750
TCAAATGTTT TTTTTTAAGG GATGGTTGAA TGAACCTCAG CATCCAAGTT     1800
TATGAATGAC AGCAGTCACA CAGTCTGTG TATATAGTTT AAGGGTAAGA     1850
GTCTTGTTGT TTATTGAGAT TGGGAAATCC ATTCTATTTT GTGAATTGGG     1900
ATAATAACAG CAGTGAATAA AGTACTTAGA AATGTGAAAA ATGAGCAGTA     1950
AAATAGATGA GATAAAGAAC TAAAGAAATT AAGAGATAGT CAATTCTTGC     2000
CTTATACCTC AGTCTATTCT GTAAAATTTT TAAAGATATA TGCATACCTG     2050
GATTTCCTTG GCTTCTTTGA GAATGTAAGA GAAATTAAAT CTGAATAAAG     2100
AATTCTTCCT GTTCACTGGC TCTTTCTTTC TCCATGCACT GAGCATCTGC     2150
TTTTTGGAAG GCCCTGGGTT AGTAGTGGAG ATGCTAAGGT AAGCCAGACT     2200

```

SUBSTITUTE SHEET

CATACCCACC	CATAGGGTCG	TAGAGTCTAG	GAGCTGCAGT	CACGTAATCG	2250
AGGTGGCAAG	ATGTCCTCTA	AAGATGTAGG	GAAAAGTGAG	AGAGGGGTGA	2300
GGGTGTGGGG	CTCCGGGTGA	GAGTGGTGGA	GTGTCAATGC	CCTGAGCTGG	2350
GGCATTTTGG	GCTTTGGGAA	ACTGCAGTTC	CTTCTGGGGG	AGCTGATTGT	2400
AATGATCTTG	GGTGGATCC				2418

**SUBSTITUTE SHEET**

71

- (2) INFORMATION FOR SEQUENCE ID NO: 8:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 5724 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
- (A) NAME/KEY: MAGE-1 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCCCGGGCAC	CACTGGCATC	CCTCCCCCTA	CCACCCCCAA	TCCCTCCCTT	50
TACGCCACCC	ATCCAAACAT	CTTCACGCTC	ACCCCCAGCC	CAAGCCAGGC	100
AGAATCCGGT	TCCACCCCTG	CTCTCAACCC	AGGGAAGCCC	AGGTGCCCAG	150
ATGTGACGCC	ACTGACTTGA	GCATTAGTGG	TTAGAGAGAA	GCGAGGTTTT	200
CGGTCTGAGG	GGCGGCTTGA	GATCGGTGGA	GGGAAGCGGG	CCCAGCTCTG	250
TAAGGAGGCA	AGGTGACATG	CTGAGGGAGG	ACTGAGGACC	CACTTACCCC	300
AGATAGAGGA	CCCCAAATAA	TCCCTTCATG	CCAGTCCTGG	ACCATCTGGT	350
GGTGGACTTC	TCAGGCTGGG	CCACCCCCAG	CCCCCTTGCT	GCTTAAACCA	400
CTGGGGACTC	GAAGTCAGAG	CTCCGTGTGA	TCAGGGAAGG	GCTGCTTAGG	450
AGAGGGCAGC	GTCCAGGCTC	TGCCAGACAT	CATGCTCAGG	ATTCTCAAGG	500
AGGGCTGAGG	GTCCCTAAGA	CCCCACTCCC	GTGACCCAAC	CCCCACTCCA	550
ATGCTCACTC	CCGTGACCCA	ACCCCTCTT	CATTGTCATT	CCAACCCCCA	600
CCCCACATCC	CCCACCCCAT	CCCTCAACCC	TGATGCCCAT	CCGCCCAGCC	650
ATTCCACCTT	CACCCCCACC	CCCACCCCCA	CGCCCACTCC	CACCCCCACC	700
CAGGCAGGAT	CCGGTTCCCG	CCAGGAAACA	TCCGGGTGCC	CGGATGTGAC	750
GCCACTGACT	TGCGCATTGT	GGGGCAGAGA	GAAGCGAGGT	TTCCATTCTG	800
AGGGACGGCG	TAGAGTTCGG	CCGAAGGAAC	CTGACCCAGG	CTCTGTGAGG	850
AGGCAAGGTG	AGAGGCTGAG	GGAGGACTGA	GGACCCCGCC	ACTCCAAATA	900
GAGAGCCCCA	AATATTCCAG	CCCCGCCCTT	GCTGCCAGCC	CTGGCCCCACC	950
CGCGGGAAGA	CGTCTCAGCC	TGGGCTGCCC	CCAGACCCCT	GCTCCAAAAG	1000
CCTTGAGAGA	CACCAGGTTT	TTCTCCCCAA	GCTCTGGAAT	CAGAGGTTGC	1050
TGTGACCAGG	GCAGGACTGG	TTAGGAGAGG	GCAGGGCACA	GGCTCTGCCA	1100
GGCATCAAGA	TCAGCACCCA	AGAGGGAGGG	CTGTGGGCCC	CCAAGACTGC	1150
ACTCCAATCC	CCACTCCCAC	CCCATTGCGA	TTCCCATTC	CCACCCAACC	1200
CCCATCTCCT	CAGCTACACC	TCCACCCCCA	TCCCTACTCC	TACTCCGTCA	1250
CCTGACCACC	ACCCTCCAGC	CCCAGCACCA	GCCCCAACC	TTCTGCCACC	1300
TCACCCTCAC	TGCCCCCAAC	CCCACCTCA	TCTCTCTCAT	GTGCCCCACT	1350
CCCATCGCCT	CCCCATTCT	GGCAGAATCC	GGTTTGCCCC	TGCTCTCAAC	1400
CCAGGGAAGC	CCTGGTAGGC	CCGATGTGAA	ACCACTGACT	TGAACCTCAC	1450
AGATCTGAGA	GAAGCCAGGT	TCATTTAATG	GTTCTGAGGG	GCGGCTTGAG	1500
ATCCACTGAG	GGGAGTGGTT	TTAGGCTCTG	TGAGGAGGCA	AGGTGAGATG	1550
CTGAGGGAGG	ACTGAGGAGG	CACACACCCC	AGGTAGATGG	CCCCAAAATG	1600
ATCCAGTACC	ACCCCTGCTG	CCAGCCCTGG	ACCACCCGGC	CAGGACAGAT	1650
GTCTCAGCTG	GACCACCCCC	CGTCCCCTCC	CACTGCCACT	TAACCCACAG	1700
GGCAATCTGT	AGTCATAGCT	TATGTGACCG	GGGCAGGGTT	GGTCAGGAGA	1750
GGCAGGGCCC	AGGCATCAAG	GTCCAGCATC	CGCCCGGCAT	TAGGGTCAGG	1800
ACCCTGGGAG	GGAAGTGAGG	GTTCCCCACC	CACACCTGTC	TCCTCATCTC	1850
CACCGCCACC	CCACTCACAT	TCCCATACTT	ACCCCTTACC	CCCAACCTCA	1900
TCTTGTGAGA	ATCCCTGCTG	TCAACCCACG	GAAGCCACGG	GAATGGCGGC	1950
CAGGCACTCG	GATCTTGACG	TCCCCATCCA	GGGTCTGATG	GAGGGAAGGG	2000
GCTTGAACAG	GGCCTCAGGG	GAGCAGAGGG	AGGGCCCTAC	TGCGAGATGA	2050
GGGAGGCCTC	AGAGGACCCA	GCACCCTAGG	ACACCGCACC	CCTGTCTGAG	2100
ACTGAGGCTG	CCACTTCTGG	CCTCAAGAAT	CAGAACGATG	GGGACTCAGA	2150

SUBSTITUTE SHEET

72

TTGCATGGGG	GTGGGACCCA	GGCCTGCAAG	GCTTACGCGG	AGGAAGAGGA	2200
GGGAGGACTC	AGGGGACCTT	GGAATCCAGA	TCAGTGTGGA	CCTCGGCCCT	2250
GAGAGGTCCA	GGGCACGGTG	GCCACATATG	GCCCATATTT	CCTGCATCTT	2300
TGAGGTGACA	GGACAGAGCT	GTGGTCTGAG	AAGTGGGGCC	TCAGGTCAAC	2350
AGAGGGAGGA	GTTCCAGGAT	CCATATGGCC	CAAGATGTGC	CCCCCTTCATG	2400
AGGACTGGGG	ATATCCCCGG	CTCAGAAAGA	AGGGACTCCA	CACAGTCTGG	2450
CTGTCCCCTT	TTAGTAGCTC	TAGGGGGACC	AGATCAGGGA	TGGCGGTATG	2500
TTCCATTCTC	ACTTGTACCA	CAGGCAGGAA	GTTGGGGGGC	CCTCAGGGAG	2550
ATGGGGTCTT	GGGGTAAAGG	GGGGATGTCT	ACTCATGTCA	GGGAATTGGG	2600
GGTTGAGGAA	GCACAGGCGC	TGGCAGGAAT	AAAGATGAGT	GAGACAGACA	2650
AGGCTATTGG	AATCCACACC	CCAGAACCAA	AGGGGTCAGC	CCTGGACACC	2700
TCACCCAGGA	TGTGGCTTCT	TTTTCACTCC	TGTTTCCAGA	TCTGGGGCAG	2750
GTGAGGACCT	CATTCTCAGA	GGGTGACTCA	GGTCAACGTA	GGGACCCCCA	2800
TCTGGTCTAA	AGACAGAGCG	GTCCCAGGAT	CTGCCATGCG	TTCGGGTGAG	2850
GAACATGAGG	GAGGACTGAG	GGTACCCAG	GACCAGAACA	CTGAGGGAGA	2900
CTGCACAGAA	ATCAGCCCTG	CCCCTGCTGT	CACCCCAGAG	AGCATGGGCT	2950
GGGCCGTCTG	CCGAGGTCCT	TCCGTTATCC	TGGGATCATT	GATGTCAGGG	3000
ACGGGGAGGC	CTTGGTCTGA	GAAGGCTGCG	CTCAGGTCAG	TAGAGGGAGC	3050
GTCCCAGGCC	CTGCCAGGAG	TCAAGGTGAG	ATCTCTTGCT	GCCCTTCCCC	3150
CAGGACACAT	TAATTCCAAT	GAATTTTGAT	ATCTCTTGCT	GCCCTTCCCC	3200
AAGGACCTAG	GCACGTGTGG	CCAGATGTTT	GTCCCTCCT	GTCCTTCCAT	3250
TCCTTATCAT	GGATGTGAAC	TCTTGATTTG	GATTTCTCAG	ACCAGCAAAA	3300
GGGCAGGATC	CAGGCCCTGC	CAGGAAAAAT	ATAAGGGCCC	TGCGTGAGAA	3350
CAGAGGGGGT	CATCCACTGC	ATGAGAGTGG	GGATGTCACA	GAGTCCAGCC	3400
CACCCTCCTG	GTAGCACTGA	GAAGCCAGGG	CTGTGCTTGC	GGTCTGCACC	3450
CTGAGGGCCC	GTGGATTCCCT	CTTCCTGGAG	CTCCAGGAAC	CAGGCAGTGA	3500
GGCCTTGGTC	TGAGACAGTA	TCCTCAGGTC	ACAGAGCAGA	GGATGCACAG	3550
GGTGTGCCAG	CAGTGAATGT	TTGCCCTGAA	TGCACACCAA	GGGCCCCACC	3600
TGCCACAGGA	CACATAGGAC	TCCACAGAGT	CTGGCCTCAC	CTCCCTACTG	3650
TCAGTCCTGT	AGAATCGACC	TCTGCTGGCC	GGCTGTACCC	TGAGTACCCT	3700
CTCACTTCCT	CCTTCAGGTT	TTCAGGGGAC	AGGCCAACCC	AGAGGACAGG	3750
ATTCCCTGGA	GGCCACAGAG	GAGCACCAAG	GAGAAGATCT	GTAAGTAGGC	3800
CTTTGTTAGA	GTCTCCAAGG	TTCAGTTCTC	AGCTGAGGCC	TCTCACACAC	3850
TCCCTCTCTC	CCCAGGCCTG	TGGGTCTTCA	TTGCCCAGCT	CCTGCCCCACA	3900
CTCCTGCCTG	CTGCCCTGAC	GAGAGTCATC			3930
ATG TCT CTT	GAG CAG AGG	AGT CTG CAC	TGC AAG CCT	GAG GAA	3972
GCC CTT GAG	GCC CAA CAA	GAG GCC CTG	GGC CTG GTG	TGT GTG	4014
CAG GCT GCC	ACC TCC TCC	TCT CCT CTG	GTC CTG GGC	ACC	4056
CTG GAG GAG	GTG CCC ACT	GCT GGG TCA	ACA GAT CCT	CCC CAG	4098
AGT CCT CAG	GGA GCC TCC	GCC TTT CCC	ACT ACC ATC	AAC TTC	4140
ACT CGA CAG	AGG CAA CCC	AGT GAG GGT	TCC AGC AGC	CGT GAA	4182
GAG GAG GGG	CCA AGC ACC	TCT TGT ATC	CTG GAG TCC	TTG TTC	4224
CGA GCA GTA	ATC ACT AAG	AAG GTG GCT	GAT TTG GTT	GGT TTT	4266
CTG CTC CTC	AAA TAT CGA	GCC AGG GAG	CCA GTC ACA	AAG GCA	4308
GAA ATG CTG	GAG AGT GTC	ATC AAA AAT	TAC AAG CAC	TGT TTT	4350
CCT GAG ATC	TTC GGC AAA	GCC TCT GAG	TCC TTG CAG	CTG GTC	4392
TTT GGC ATT	GAC GTG AAG	GAA GCA GAC	CCC ACC GGC	CAC TCC	4434
TAT GTC CTT	GTC ACC TGC	CTA GGT CTC	TCC TAT GAT	GGC CTG	4476
CTG GGT GAT	AAT CAG ATC	ATG CCC AAG	ACA GGC TTC	CTG ATA	4518
ATT GTC CTG	GTC ATG ATT	GCA ATG GAG	GGC GGC CAT	GCT CCT	4560
GAG GAG GAA	ATC TGG GAG	GAG CTG AGT	GTG ATG GAG	GTG TAT	4602
GAT GGG AGG	GAG CAC AGT	GCC TAT GGG	GAG CCC AGG	AAG CTG	4644
CTC ACC CAA	GAT TTG GTG	CAG GAA AAG	TAC CTG GAG	TAC GGC	4686
AGG TGC CGG	ACA GTG ATC	CCG CAC GCT	ATG AGT TCC	TGT GGG	4728
GTC CAA GGG	CCC TCG CTG	AAA CCA GCT	ATG TGA		4761

SUBSTITUTE SHEET

73

AAGTCCTTGA	GTATGTGATC	AAGGTCAGTG	CAAGAGTTC	4800
GCTTTTTTCTT	CCCATCCCTG	CGTGAAGCAG	CTTTGAGAGA	4850
GGAGTCTGAG	CATGAGTTGC	AGCCAAGGCC	AGTGGGAGGG	4900
AGTGCACCTT	CCAGGGCCGC	GTCCAGCAGC	TTCCCCTGCC	4950
TGAGGCCCCAT	TCTTCACTCT	GAAGAGAGCG	GTCAGTGTTT	5000
GTTTCTGTTC	TATTGGGTGA	CTTGGAGATT	TATCTTTGTT	5050
ATTGTTCAAA	TGTTTTTTTT	TAAGGGATGG	TTGAATGAAC	5100
AAGTTTATGA	ATGACAGCAG	TCACACAGTT	CTGTGTATAT	5150
TAAGAGTCTT	GTGTTTTATT	CAGATTGGGA	AATCCATTCT	5200
TTGGGATAAT	AACAGCAGTG	GAATAAGTAC	TTAGAAATGT	5250
CAGTAAAATA	GATGAGATAA	AGAAGTAAAG	AAATTAAGAG	5300
CTTGCCCTTAT	ACCTCAGTCT	ATTCTGTAAA	ATTTTAAAG	5350
ACCTGGATTT	CCTTGGCTTC	TTTGAGAATG	TAAGAGAAAT	5400
TAAAGAATTC	TTCCTGTTCA	CTGGCTCTTT	TCTTCTCCAT	5450
TCTGCTTTTT	GGAAGGCCCT	GGGTAGTAG	TGGAGATGCT	5500
AGACTCATAC	CCACCCATAG	GGTCGTAGAG	TCTAGGAGCT	5550
AATCGAGGTG	GCAAGATGTC	CTCTAAAGAT	GTAGGGAAAA	5600
GGTGAGGGTG	TGGGGCTCCG	GGTGAGAGTG	GTGGAGTGTC	5650
GCTGGGGCAT	TTTGGGCTTT	GGGAACTGC	AGTTCCTTCT	5700
ATTGTAATGA	TCTTGGGTGG	ATCC		5724

SUBSTITUTE SHEET

74

- (2) INFORMATION FOR SEQUENCE ID NO: 9:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4157 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
- (A) NAME/KEY: MAGE-2 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```

CCCATCCAGA TCCCCATCCG GGCAGAATCC GGTTCACCCC TTGCCGTGAA      50
CCCAGGGAAG TCACGGGCCC GGATGTGACG CCACTGACTT GCACATTGGA      100
GGTCAGAGGA CAGCGAGATT CTCGCCCTGA GCAACGGCCT GACGTCGGCG      150
GAGGGAAGCA GGCGCAGGCT CCGTGAGGAG GCAAGGTAAG ACGCCGAGGG      200
AGGACTGAGG CGGGCCTCAC CCCAGACAGA GGGCCCCCAA TTAATCCAGC      250
GCTGCCTCTG CTGCCGGGCC TGGACCACCC TGCAGGGGAA GACTTCTCAG      300
GCTCAGTCGC CACCACCTCA CCCCGCCACC CCCC GCCGCT TTAACCGCAG      350
GGAACCTCTG CGTAAGAGCT TTGTGTGACC AGGGCAGGGC TGGTTAGAAG      400
TGCTCAGGGC CCAGACTCAG CCAGGAATCA AGGTCAGGAC CCAAGAGGG      450
GACTGAGGGC AACCACCCCC CTACCCTCAC TACCAATCCC ATCCCCCAAC      500
ACCAACCCCA CCCCATCCC TCAAACACCA ACCCACCCC CAAACCCCAT      550
TCCCATCTCC TCCCCACCA CCATCCTGGC AGAATCCGGC TTTGCCCTG      600
CAATCAACCC ACGGAAGCTC CGGGAATGGC GGCCAAGCAC GCGGATCCTG      650
ACGTTACAT GTACGGCTAA GGGAGGGAAG GGGTTGGGTC TCGTGAGTAT      700
GGCCTTTGGG ATGCAGAGGA AGGGCCAGG CCTCTGGAA GACAGTGGAG      750
TCCTTAGGGG ACCCAGCATG CCAGGACAGG GGGCCACTG TACCCCTGTC      800
TCAAACCTGAG CCACCTTTTC ATTACGCCGA GGGAATCCTA GGGATGCAGA      850
CCCACTTCAG GGGGTTGGGG CCCAGCCTGC GAGGAGTCAA GGGGAGGAAG      900
AAGAGGGAGG ACTGAGGGGA CTTGGAGTC CAGATCAGTG GCAACCTTGG      950
GCTGGGGGAT CCTGGGCACA GTGGCCGAAT GTGCCCCGTG CTCATTGCAC      1000
CTTCAGGGTG ACAGAGAGTT GAGGGCTGTG GTCTGAGGGC TGGGACTTCA      1050
GGTCAGCAGA GGGAGGAATC CCAGGATCTG CCGGACCCAA GGTGTGCCCC      1100
CTTCATGAGG ACTCCCATA CCCC CGGCC AGAAAGAAGG GATGCCACAG      1150
AGTCTGGAAG TAAATTGTTT TTAGCTCTGG GGAACCTGA TCAGGGATGG      1200
CCCTAAGTGA CAATCTCATT TGTACCACAG GCAGGAGGTT GGGGAACCCCT      1250
CAGGGAGATA AGGTGTTGGT GTAAAGAGGA GCTGTCTGCT CATTTCAGGG      1300
GGTTCCCCCT TGAGAAAGGG CAGTCCCTGG CAGGAGTAAA GATGAGTAAC      1350
CCACAGGAGG CCATCATAAC GTTACCCTA GAACCAAAGG GGTGAGCCCT      1400
GGACAACGCA CGTGGGGTAA CAGGATGTGG CCCCTCCTCA CTTGTCTTTC      1450
CAGATCTCAG GGAGTTGATG ACCTTGTTTT CAGAAGGTGA CTCAGTCAAC      1500
ACAGGGGCCC CTCTGGTCGA CAGATGCAGT GGTCTAGGA TCTGCCAAGC      1550
ATCCAGGTGG AGAGCCTGAG GTAGGATTGA GGGTACCCCT GGGCCAGAAT      1600
GCAGCAAGGG GGCCCCATAG AAATCTGCCC TGCCCCCTGCG GTTACTTCAG      1650
AGACCCTGGG CAGGGCTGTC AGCTGAAGTC CCTCCATTAT CTGGGATCTT      1700
TGATGTCAGG GAAGGGGAGG CCTTGGTCTG AAGGGGCTGG AGTCAGGTCA      1750
GTAGAGGGAG GGTCTCAGGC CCTGCCAGGA GTGGACGTGA GGACCAAGCG      1800
GACTCGTCAC CCAGGACACC TGGACTCAA TGAATTTGAC ATCTCTCGTT      1850
GTCCCTTCGCG GAGGACCTGG TCACGTATGG CCAGATGTGG GTCCCCTCTA      1900
TCTCCTTCTG TACCATATCA GGGATGTGAG TTCTTGACAT GAGAGATTCT      1950
CAAGCCAGCA AAAGGGTGGG ATTAGGCCCT ACAAGGAGAA AGGTGAGGGC      2000
CCTGAGTGAG CACAGAGGGG ACCCTCCACC CAAGTAGAGT GGGGACCTCA      2050
CGGAGTCTGG CCAACCCTGC TGAGACTTCT GGAATCCGT GGCTGTGCTT      2100
GCAGTCTGCA CACTGAAGGC CCGTGCATTC CTCTCCAGG AATCAGGAGC      2150

```

SUBSTITUTE SHEET

TCCAGGAACC	AGGCAGTGAG	GCCTTGGTCT	GAGTCAGTGC	CTCAGGTCAC	2200
AGAGCAGAGG	GGACGCAGAC	AGTGCCAACA	CTGAAGGTTT	GCCTGGAATG	2250
CACACCAAGG	GCCCCACCCG	CCCAGAACAA	ATGGGACTCC	AGAGGGCCTG	2300
GCCTCACCCCT	CCCTATTCTC	AGTCCTGCAG	CCTGAGCATG	TGCTGGCCGG	2350
CTGTACCCTG	AGGTGCCCTC	CCACTTCCTC	CTTCAGGTTC	TGAGGGGGAC	2400
AGGCTGACAA	GTAGGACCCG	AGGCACTGGA	GGAGCATTGA	AGGAGAAGAT	2450
CTGTAAGTAA	GCCTTTGTCA	GAGCCTCCAA	GGTTCAGTTC	AGTTCTCACC	2500
TAAGGCCTCA	CACACGCTCC	TTCTCTCCCC	AGGCCTGTGG	GTCTTCATTG	2550
CCCAGCTCCT	GCCCGCACTC	CTGCCTGCTG	CCCTGACCAG	AGTCATC	2597
ATG CCT CTT	GAG CAG AGG	AGT CAG CAC	TGC AAG CCT	GAA GAA	2639
GGC CTT GAG	GCC CGA GGA	GAG GCC CTG	GGC CTG GTG	GGT GCG	2681
CAG GCT CCT	GCT ACT GAG	GAG CAG CAG	ACC GCT TCT	TCC TCT	2723
TCT ACT CTA	GTG GAA GTT	ACC CTG GGG	GAG GTG CCT	GCT GCC	2765
GAC TCA CCG	AGT CCT CCC	CAC AGT CCT	CAG GGA GCC	TCC AGC	2807
TTC TCG ACT	ACC ATC AAC	TAC ACT CTT	TGG AGA CAA	TCC GAT	2849
GAG GGC TCC	AGC AAC CAA	GAA GAG GAG	GGG CCA AGA	ATG TTT	2891
CCC GAC CTG	GAG TCC GAG	TTC CAA GCA	GCA ATC AGT	AGG AAG	2933
ATG GTT GAG	TTG GTT CAT	TTT CTG CTC	CTC AAG TAT	CGA GCC	2975
AGG GAG CCG	GTC ACA AAG	GCA GAA ATG	CTG GAG AGT	GTC CTC	3017
AGA AAT TGC	CAG GAC TTC	TTT CCC GTG	ATC TTC AGC	AAA GCC	3059
TCC GAG TAC	TTG CAG CTG	GTC TTT GGC	ATC GAG GTG	GTG GAA	3101
GTG GTC CCC	ATC AGC CAC	TTG TAC ATC	CTT GTC ACC	TGC CTG	3143
GGC CTC TCC	TAC GAT GGC	CTG CTG GGC	GAC AAT CAG	GTC ATG	3185
CCC AAG ACA	GGC CTC CTG	ATA ATC GTC	CTG GCC ATA	ATC GCA	3227
ATA GAG GGC	GAC TGT GCC	CCT GAG GAG	AAA ATC TGG	GAG GAG	3269
CTG AGT ATG	TTG GAG GTG	TTT GAG GGG	AGG GAG GAC	AGT GTC	3311
TTC GCA CAT	CCC AGG AAG	CTG CTC ATG	CAA GAT CTG	GTG CAG	3353
GAA AAC TAC	CTG GAG TAC	CGG CAG GTG	CCC GGC AGT	GAT CCT	3395
GCA TGC TAC	GAG TTC CTG	TGG GGT CCA	AGG GCC CTC	ATT GAA	3437
ACC AGC TAT	GTG AAA GTC	CTG CAC CAT	ACA CTA AAG	ATC GGT	3479
GGA GAA CCT	CAC ATT TCC	TAC CCA CCC	CTG CAT GAA	CGG GCT	3521
TTG AGA GAG	GGA GAA GAG	TGA			3542
GTCTCAGCAC	ATGTTGCAGC	CAGGGCCAGT	GGGAGGGGGT	CTGGGCCAGT	3592
GCACCTTCCA	GGGCCCCATC	CATTAGCTTC	CACTGCCCTCG	TGTGATATGA	3642
GGCCCATTC	TGCCTCTTTG	AAGAGAGCAG	TCAGCATTCT	TAGCAGTGAG	3692
TTTCTGTTCT	GTTGGATGAC	TTTGAGATTT	ATCTTTCTTT	CCTGTTGGAA	3742
TTGTTCAAAT	GTTCCTTTTA	ACAAATGGTT	GGATGAACTT	CAGCATCCAA	3792
GTTTATGAAT	GACAGTAGTC	ACACATAGTG	CTGTTTATAT	AGTTTAGGGG	3842
TAAGAGTCCT	GTTTTTTATT	CAGATTGGGA	AATCCATTCC	ATTTTGTGAG	3892
TTGTACATA	ATAACAGCAG	TGGAATATGT	ATTTGCCTAT	ATTGTGAACG	3942
AATTAGCAGT	AAAATACATG	ATACAAGGAA	CTCAAAAGAT	AGTTAATTCT	3992
TGCCTTATAC	CTCAGTCTAT	TATGTAAAAT	TAAAAATATG	TGTATGTTTT	4042
TGCTTCTTTG	AGAATGCAAA	AGAAATTAAA	TCTGAATAAA	TTCTTCCTGT	4092
TCACTGGCTC	ATTTCTTTAC	CATTCACTCA	GCATCTGCTC	TGTGGAAGGC	4142
CCTGGTAGTA	GTGGG				4157

SUBSTITUTE SHEET

76

## (2) INFORMATION FOR SEQUENCE ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 662 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-21 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATCCCCAT	GGATCCAGGA	AGAATCCAGT	TCCACCCCTG	CTGTGAACCC	50
AGGGAAGTCA	CGGGGCCGGA	TGTGACGCCA	CTGACTTGCG	CGTTGGAGGT	100
CAGAGAACAG	CGAGATTCTC	GCCCTGAGCA	ACGGCCTGAC	GTCGGCGGAG	150
GGAAGCAGGC	GCAGGCTCCG	TGAGGAGGCA	AGGTAAGATG	CCGAGGGAGG	200
ACTGAGGCGG	GCCTCACCCC	AGACAGAGGG	CCCCCAATAA	TCCAGCGCTG	250
CCTCTGCTGC	CAGGCCTGGA	CCACCCTGCA	GGGGAAGACT	TCTCAGGCTC	300
AGTCGCCACC	ACCTCACCCC	GCCACCCCCC	GCCGCTTTAA	CCGCAGGGAA	350
CTCTGGTGTA	AGAGCTTTGT	GTGACCAGGG	CAGGGCTGGT	TAGAAGTGCT	400
CAGGGCCCAG	ACTCAGCCAG	GAATCAAGGT	CAGGACCCCA	AGAGGGGACT	450
GAGGGTAACC	CCCCCGCACC	CCCACCACCA	TTCCCATCCC	CCAACACCAA	500
CCCCACCCCC	ATCCCCCAAC	ACCAAACCCA	CCACCATCGC	TCAAACATCA	550
ACGGCACCCC	CAAACCCCGA	TTCCCATCCC	CACCCATCCT	GGCAGAATCG	600
GAGCTTTGCC	CCTGCAATCA	ACCCACGGAA	GCTCCGGGAA	TGGCGGCCAA	650
GCACGCGGAT	CC				662

SUBSTITUTE SHEET



## (2) INFORMATION FOR SEQUENCE ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1640 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

## (ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```

GCCGCGAGGG AAGCCGGCCC AGGCTCGGTG AGGAGGCAAG GTTCTGAGGG      50
GACAGGCTGA CCTGGAGGAC CAGAGGCCCC CGGAGGAGCA CTGAAGGAGA      100
AGATCTGCCA GTGGGTCTCC ATTGCCCAGC TCCTGCCCAC ACTCCCGCCT      150
GTTGCCCTGA CCAGAGTCAT C                                171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA      213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG      255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT      297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC      339
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC      381
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT      423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC      465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG      507
GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC      549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC      591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT      633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA      675
GGC GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG      717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG      759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTC GCC ATA ATC GCA      801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG      843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG      885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG      927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT      969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA     1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT     1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT     1095
TTG AGA GAG GGG GAA GAG TGA                                1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT     1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA     1216
GGCCCATTTCT TCACTCTTTG AAGCGAGCAG TCAGCATTCT TAGTAGTGGG     1266
TTTCTGTTCT GTTGATGAC TTTGAGATTA TTCTTTGTTT CCTGTTGGAG     1316
TTGTTCAAAT GTTCCTTTTA ACGGATGGTT GAATGAGCGT CAGCATCCAG     1366
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGAG     1416
TAAGAGTCTT GttTTTTACT CAAATTGGGA AATCCATTCC ATTTTGTGAA     1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC     1516
GAATTAGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG     1566
ATTCTTGCCT TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA     1616
ACCAGGATTT CCTTGACTTC TTTG                                1640

```

## SUBSTITUTE SHEET

- (2) INFORMATION FOR SEQUENCE ID NO: 12:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 943 base pairs
    - (B) TYPE: nucleic acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: genomic DNA
  - (ix) FEATURE:
    - (A) NAME/KEY: MAGE-31 gene
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA	CCCCAGTAGA	GTGGGGACCT	CACAGAGTCT	GGCCAACCCT	50
CCTGACAGTT	CTGGGAATCC	GTGGCTGCGT	TTGCTGTCTG	CACATTGGGG	100
GCCCGTGGAT	TCCTCTCCCA	GGAATCAGGA	GCTCCAGGAA	CAAGGCAGTG	150
AGGACTTGGT	CTGAGGCAGT	GTCCTCAGGT	CACAGAGTAG	AGGGGGCTCA	200
GATAGTGCCA	ACGGTGAAGG	TTTGCTTGG	ATTCAAACCA	AGGGCCCCAC	250
CTGCCCCAGA	ACACATGGAC	TCCAGAGCGC	CTGGCCTCAC	CCTCAATACT	300
TTCACTCCTG	CAGCCTCAGC	ATGCGCTGGC	CGGATGTACC	CTGAGGTGCC	350
CTCTCACTTC	CTCCTTCAGG	TTCTGAGGGG	ACAGGCTGAC	CTGGAGGACC	400
AGAGGCCCCC	GGAGGAGCAC	TGAAGGAGAA	GATCTGTAAG	TAAGCCTTTG	450
TTAGAGCCTC	CAAGGTTCCA	TTCAGTACTC	AGCTGAGGTC	TCTCACATGC	500
TCCCTCTCTC	CCCAGGCCAG	TGGGTCTCCA	TTGCCCAGCT	CCTGCCCCACA	550
CTCCCGCCTG	TTGCCCTGAC	CAGAGTCATC			580
ATG CCT CTT	GAG CAG AGG	AGT CAG CAC	TGC AAG CCT	GAA GAA	622
GGC CTT GAG	GCC CGA GGA	GAG GCC CTG	GGC CTG GTG	GGT GCG	664
CAG GCT CCT	GCT ACT GAG	GAG CAG GAG	GCT GCC TCC	TCC TCT	706
TCT AGT GTA	GTT GAA GTC	ACC CTG GGG	GAG GTG CCT	GCT GCC	748
GAG TCA CCA	GAT CCT CCC	CAG AGT CCT	CAG GGA GCC	TCC AGC	790
CTC CCC ACT	ACC ATG AAC	TAC CCT CTC	TGG AGC CAA	TCC TAT	832
GAG GAC TCC	AGC AAC CAA	GAA GAG GAG	GGG CCA AGC	ACC TTC	874
CCT GAC CTG	GAG TCT GAG	TTC CAA GCA	GCA CTC AGT	AGG AAG	916
GTG GCC AAG	TTG GTT CAT	TTT CTG CTC			943

SUBSTITUTE SHEET

79

## (2) INFORMATION FOR SEQUENCE ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2531 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-4 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG      50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC      100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG      150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT      200
TGGTCTGAGA CAGTGTCTCT AGGTTACAGA GCAGAGGATG CACAGGCTGT      250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA      300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT      350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA      400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC      450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT      500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC      550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACCTCTG      600
CCTGCTGCCC TGACCAGAGT CATC                                     624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA      708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC      750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT      792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT      834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC      876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC      918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC      960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA     1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC     1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA     1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG     1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC     1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC     1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT     1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG     1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT     1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG     1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT     1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT     1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC     1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA     1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA                     1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC     1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC     1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT     1728
TCTATTTTGT TGGATGACTT GGAGATTAT CTCTGTTTCC TTTTACAATT     1778
TTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT     1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG     1878
AGTCTTGTTT TTTATTGAGA TTGGGAAATC CGTCTATTT TGTGAATTTG     1928

```

SUBSTITUTE SHEET

80

GGACATAATA	ACAGCAGTGG	AGTAAGTATT	TAGAAGTGTG	AATTCACCGT	1978
GAAATAGGTG	AGATAAATTA	AAAGATACTT	AATCCCCGCC	TTATGCCTCA	2028
GTCTATTCTG	TAAAATTTAA	AAATATATAT	GCATACCTGG	ATTTCCCTTG	2078
CTTCGTGAAT	GTAAGAGAAA	TTAAATCTGA	ATAAATAATT	CTTTCTGTTA	2128
ACTGGCTCAT	TTCTTCTCTA	TGCACTGAGC	ATCTGCTCTG	TGGAAGGCCC	2178
AGGATTAGTA	GTGGAGATAC	TAGGGTAAGC	CAGACACACA	CCTACCGATA	2228
GGGTATTAAG	AGTCTAGGAG	CGCGGTCATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAAGAT	GTAGGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAAT	TCCCTGTGTG	GGGCCTTTTG	GGCTTTGGGA	2378
AACTGCATTT	TCTTCTGAGG	GATCTGATTC	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGGAAA	GCCCAGATTG	GAAAAGTTGC	2478
TCTGAGCAGT	TCCTTTGTGA	CAATGGATGA	ACAGAGAGGA	GCCTCTACCT	2528
GGG					2531

SUBSTITUTE SHEET

## (2) INFORMATION FOR SEQUENCE ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2531 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-41 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG      50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC      100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG      150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT      200
TGGTCTGAGA CAGTGTCTCT AGGTTACAGA GCAGAGGATG CACAGGCTGT      250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA      300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT      350
CCTGCAGAAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA      400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC      450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT      500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC      550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACCTTG      600
CCTGCTGCCC TGAGCAGAGT CATC                                     624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG      708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC      750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT      792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT      834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC      876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC      918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC      960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA     1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC     1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA     1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG     1128
GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC     1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC     1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT     1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG     1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT     1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG     1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT     1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT     1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC     1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA     1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA                       1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC     1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC     1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT     1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT     1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT     1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG     1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG     1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT     1978

```

SUBSTITUTE SHEET

82

GAAATAGGTG	AGATAAATTA	AAAGATACTT	AATTCCCGCC	TTATGCCTCA	2028
GTCTATTCTG	TAAAATTTAA	AAATATATAT	GCATACCTGG	ATTCCTTGG	2078
CTTCGTGAAT	GTAAGAGAAA	TTAAATCTGA	ATAAATAATT	CTTTCTGTTA	2128
ACTGGCTCAT	TTCTTCTCTA	TGCACTGAGC	ATCTGCTCTG	TGGAAGGCC	2178
AGGATTAGTA	GTGGAGATAC	TAGGGTAAGC	CAGACACACA	CCTACCGATA	2228
GGGTATTAAG	AGTCTAGGAG	CGCGGTCATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAAGAT	GTAGGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAAT	TCCCTGTGTG	GGGCCTTTTG	GGCTTTGGGA	2378
AACTCCATTT	TCTTCTGAGG	GATCTGATTC	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGAAAA	GCCCAGATTG	GAAAAGTTGC	2478
TCTGAGCGGT	TCCTTTGTGA	CAATGGATGA	ACAGAGAGGA	GCCTCTACCT	2528
GGG					2531

SUBSTITUTE SHEET

83

## (2) INFORMATION FOR SEQUENCE ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1068 bas pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

## (ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

G GGG CCA AGC ACC TCG CCT GAC GCA GAG TCC TTG TTC CGA	40
GAA GCA CTC AGT AAC AAG GTG GAT GAG TTG GCT CAT TTT CTG	82
CTC CGC AAG TAT CGA GCC AAG GAG CTG GTC ACA AAG GCA GAA	124
ATG CTG GAG AGA GTC ATC AAA AAT TAC AAG CGC TGC TTT CCT	166
GTG ATC TTC GGC AAA GCC TCC GAG TCC CTG AAG ATG ATC TTT	208
GGC ATT GAC GTG AAG GAA GTG GAC CCC GCC AGC AAC ACC TAC	250
ACC CTT GTC ACC TGC CTG GGC CTT TCC TAT GAT GGC CTG CTG	292
GGT AAT AAT CAG ATC TTT CCC AAG ACA GGC CTT CTG ATA ATC	334
GTC CTG GGC ACA ATT GCA ATG GAG GGC GAC AGC GCC TCT GAG	376
GAG GAA ATC TGG GAG GAG CTG GGT GTG ATG GGG GTG TAT GAT	418
GGG AGG GAG CAC ACT GTC TAT GGG GAG CCC AGG AAA CTG CTC	460
ACC CAA GAT TGG GTG CAG GAA AAC TAC CTG GAG TAC CGG CAG	502
GTA CCC GGC AGT AAT CCT GCG CGC TAT GAG TTC CTG TGG GGT	544
CCA AGG GCT CTG GCT GAA ACC AGC TAT GTG AAA GTC CTG GAG	586
CAT GTG GTC AGG GTC AAT GCA AGA GTT CGC ATT GCC TAC CCA	628
TCC CTG CGT GAA GCA GCT TTG TTA GAG GAG GAA GAG GGA GTC	670
TGAGCATGAG TTGCAGCCAG GGCTGTGGGG AAGGGGCAGG GCTGGGCCAG	720
TGCATCTAAC AGCCCTGTGC AGCAGCTTCC CTTGCCTCGT GTAACATGAG	770
GCCCATTCTT CACTCTGTTT GAAGAAAATA GTCAGTGTTT TTAGTAGTGG	820
GTTTCTATTT TGTGATGA CTTGGAGATT TATCTCTGTT TCCTTTTACA	870
ATTGTTGAAA TGTCCTTTT AATGGATGGT TGAATTAAC TCAGCATCCA	920
AGTTTATGAA TCGTAGTTAA CGTATATTGC TGTTAATATA GTTTAGGAGT	970
AAGAGTCTTG TTTTATTTC AGATTGGGAA ATCCGTTCTA TTTTGTGAAT	1020
TTGGGACATA ATAACAGCAG TGGAGTAAGT ATTTAGAAGT GTGAATTC	1068

SUBSTITUTE SHEET

84

- (2) INFORMATION FOR SEQUENCE ID NO: 16:  
 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 2226 base pairs  
     (B) TYPE: nucleic acid  
     (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: genomic DNA  
 (ix) FEATURE:  
     (A) NAME/KEY: MAGE-5 gene  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```

GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG      50
GGGACCATTG ACCCAAGAG GGTGGAGACC TCACAGATTG CAGCCTACCC      100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG      150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT      200
TGGTCTGAGG CCGTGCCCTC AGGTACAGA GCAGAGGAGA TGCAGACGTC      250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC      300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG      350
TCAGTCCCTG AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT      400
CTCACTTTTT CTTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG      450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG      500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCACT TTTTAGCTGA      550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC      600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC          644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      684
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG      728
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA      770
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA      812
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC      854
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG      896
TGG CTG ACT TGA          908
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCTGGT CACAAAGGCA      958
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT     1008
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA     1058
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA     1108
CTCCTATGAT GGCCTGCTGG TTGATAATAA TCAGATCATG CCCAAGACGG      1158
GCCTCCTGAT AATCGTCTTG GGCATGATTG CAATGGAGGG CAAATGCGTC      1208
CCTGAGGAGA AAATCTGGGA GGAGCTGAGT GTGATGAAGG TGTATGTTGG      1258
GAGGGAGCAC AGTGTCTGTG GGGAGCCCAG GAAGCTGCTC ACCCAAGATT      1308
TGGTGCAGGA AACTACCTG GAGTACCGGC AGGTGCCCAG CAGTGATCCC      1358
ATATGCTATG AGTTACTGTG GGGTCCAAGG GCACTCGCTG CTTGAAAGTA      1408
CTGGAGCACG TGGTCAGGGT CAATGCAAGA GTTCTCATTT CCTACCCATC      1458
CCTGCGTGAA GCAGCTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG      1508
CTGCAGCCAG GGCCACTGCG AGGGGGGCTG GGCCAGTGCA CCTTCCAGGG      1558
TCCCGTCCAG TAGTTTCCCC TGCCTTAATG TGACATGAGG CCCATTCTTC      1608
TCTCTTTGAA GAGAGCAGTC AACATTCTTA GTAGTGGGTT TCTGTTCTAT      1658
TGGATGACTT TGAGATTGT CTTTGTTCCT TTTTGAATT GTTCAAATGT      1708
TTCTTTTAAT GGGTGGTTGA ATGAACTTCA GCATTCAAAT TTATGAATGA      1758
CAGTAGTCAC ACATAGTGCT GTTTATATAG TTTAGGAGTA AGAGTCTTGT      1808
TTTTTATTCA GATTGGGAAA TCCATTCCAT TTTGTGAATT GGGACATAGT      1858
TACAGCAGTG GAATAAGTAT TCATTTAGAA ATGTGAATGA GCAGTAAAC      1908
TGATGACATA AAGAAATTAA AAGATATTTA ATTCTTGCTT ATACTCAGTC      1958
TATTCGGTAA AATTTTTTTT AAAAAATGTG CATACCTGGA TTTCTTGGC      2008
TTCTTTGAGA ATGTAAGACA AATTAAATCT GAATAAATCA TTCTCCCTGT      2058

```

SUBSTITUTE SHEET



85

TC	ACTGGCTC	ATTTATTCTC	TATGCACTGA	GCATTTGCTC	TGTGGAAGGC	2108
CCT	GGGTAA	TAGTGGAGAT	GCTAAGGTAA	GCCAGACTCA	CCCCTACCCA	2158
CAG	GGTAGTA	AAGTCTAGGA	GCAGCAGTCA	TATAATTAAG	GTGGAGAGAT	2208
GCC	CTCTAAG	ATGTAGAG				2226

**SUBSTITUTE SHEET**

86

- (2) INFORMATION FOR SEQUENCE ID NO: 17:  
 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 2305 base pairs  
     (B) TYPE: nucleic acid  
     (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: genomic DNA  
 (ix) FEATURE:  
     (A) NAME/KEY: MAGE-51 gene  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```

GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG      50
GGGACCAATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC      100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG      150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT      200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC      250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC      300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG      350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT      400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG      450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG      500
ATCTGTAAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCACT TTTTAGCTGA      550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC      600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC          644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      686
GGC CTT GAC ACC CAA GAA GAG CCC TGG GCC TGG TGG GTG TGC      728
AGG CTG CCA CTA CTG AGG AGC AGG AGG CTG TGT CCT CCT CCT      770
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG      812
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA      854
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA      896
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC      938
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG      980
TGG CTG ACT TGA          992
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCCGGT CACAAAGGCA      1042
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT      1092
CTTCGGCAAA GCCTCCGAGT CCTTGACGCT GGTCTTTGGC ATTGACGTGA      1142
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA      1192
CTCCTATGAT GGCCTGGTGG TTTAATCAGA TCATGCCCAA GACGGGCCCTC      1242
CTGATAATCG TCTTGGGCAT GATTGCAATG GAGGGCAAAT GCGTCCCTGA      1292
GGAGAAAATC TGGGAGGAGC TGGGTGTGAT GAAGGTGTAT GTTGGGAGGG      1342
AGCACAGTGT CTGTGGGGAG CCCAGGAAGC TGCTCACCCA AGATTTGGTG      1392
CAGGAAAACCT ACCTGGAGTA CCGCAGGTGC CCAGCAGTGA TCCCATATGC      1442
TATGAGTTAC TGTGGGGTCC AAGGGCACTC GCTGCTTGAA AGTACTGGAG      1492
CACGTGGTCA GGGTCAATGC AAGAGTTCTC ATTTCCCTACC CATCCCTGCA      1542
TGAAGCAGCT TTGAGAGAGG AGGAAGAGGG AGTCTGAGCA TGAGCTGCAG      1592
CCAGGGCCAC TGCAGGGGGG GCTGGGCCAG TGCACCTTCC AGGGCTCCGT      1642
CCAGTAGTTT CCCCTGCCTT AATGTGACAT GAGGCCCATT CTTCTCTCTT      1692
TGAAGAGAGC AGTCAACATT CTTAGTAGTG GGTTCCTGTT CTATTGGATG      1742
ACTTTGAGAT TTGTCTTTGT TTCCTTTTGG AATTGTTCAA ATGTTCCCTT      1792
TAATGGGTGG TTGAATGAAC TTCAGCATTG AAATTTATGA ATGACAGTAG      1842
TCACACATAG TGCTGTTTAT ATAGTTTAGG AGTAAGAGTC TTGTTTTTTA      1892
TTCAGATTGG GAAATCCATT CCATTTTGTG AATTGGGACA TAGTTACAGC      1942
AGTGGAATAA GTATTCATTT AGAAATGTGA ATGAGCAGTA AAAGTATGTA      1992
GATAAAGAAA TTAAAAGATA TTTAATTCTT GCCTTATACT CAGTCTATTC      2042

```

UBSTITUTE SHEET

87

GGTAAAATT	TTTTTTAAA	ATGTGCATAC	CTGGATTTC	TTGGCTTCT	2092
TGAGAAATGTA	AGACAAATTA	AATCTGAATA	AATCATTCTC	CCTGTTCACT	2142
GGCTCATTTA	TTCTCTATGC	ACTGAGCATT	TGCTCTGTGG	AAGGCCCTGG	2192
GTTAATAGTG	GAGATGCTAA	GGTAAGCCAG	ACTCACCCT	ACCCACAGGG	2242
TAGTAAAGTC	TAGGAGCAGC	AGTCATATAA	TTAAGGTGGA	GAGATGCCCT	2292
CTAAGATGTA	GAG				2305

SUBSTITUTE SHEET

88

- (2) INFORMATION FOR SEQUENCE ID NO: 18:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 225 base pairs
    - (B) TYPE: nucleic acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: cDNA
  - (ix) FEATURE:
    - (A) NAME/KEY: MAGE-6 gene
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TAT	TTC	TTT	CCT	GTG	ATC	TTC	AGC	AAA	GCT	TCC	GAT	TCC	TTG	42
CAG	CTG	GTC	TTT	GGC	ATC	GAG	CTG	ATG	GAA	GTG	GAC	CCC	ATC	84
GGC	CAC	GTG	TAC	ATC	TTT	GCC	ACC	TGC	CTG	GGC	CTC	TCC	TAC	126
GAT	GGC	CTG	CTG	GGT	GAC	AAT	CAG	ATC	ATG	CCC	AGG	ACA	GGC	168
TTC	CTG	ATA	ATC	ATC	CTG	GCC	ATA	ATC	GCA	AGA	GAG	GGC	GAC	210
TGT	GCC	CCT	GAG	GAG										225

SUBSTITUTE SHEET

## (2) INFORMATION FOR SEQUENCE ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1947 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-7 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA	ACAAGGGCCC	CACACTCCCC	AGAACACAAG	GGACTCCAGA	50
GAGCCCAGCC	TCACCTTCCC	TACTGTCACT	CCTGCAGCCT	CAGCCTCTGC	100
TGGCCGGCTG	TACCCTGAGG	TGCCCTCTCA	CTTCCTCCTT	CAGGTTCTCA	150
GCGGACAGGC	CGGCCAGGAG	GTCAGAAGCC	CCAGGAGGCC	CCAGAGGAGC	200
ACCGAAGGAG	AAGATCTGTA	AGTAGGCCTT	TGTTAGGGCC	TCCAGGGCGT	250
GGTTCACAAA	TGAGGCCCTT	CACAAGCTCC	TTCTCTCCCC	AGATCTGTGG	300
GTTCTCTCCC	ATCGCCCAGC	TGCTGCCCCG	ACTCCAGCCT	GCTGCCCTGA	350
CCAGAGTCAT	CATGTCTTCT	GAGCAGAGGA	GTCAGCACTG	CAAGCCTGAG	400
GATGCCTTGA	GGCCCAAGGA	CAGGAGGCTC	TGGGCCTGGT	GGGTGCGCAG	450
GCTCCCGCCA	CCGAGGAGCA	CGAGGCTGCC	TCCTCCTTCA	CTCTGATTGA	500
AGGCACCCTG	GAGGAGGTGC	CTGCTGCTGG	GTCCCCCAGT	CCTCCCCTGA	550
GTCTCAGGGT	TCCTCCTTTT	CCCTGACCAT	CAGCAACAAC	ACTCTATGGA	600
GCCAATCCAG	TGAGGGCACC	AGCAGCCGGG	AAGAGGAGGG	GCCAACCACC	650
TAGACACACC	CCGCTCACCT	GCGCTCCTTG	TTCCA		685
ATG GGA AGG TGG CTG AGT TGG TTC GCT TCC TGC TGC ACA AGT					727
ATC GAG TCA AGG AGC TGG TCA CAA AGG CAG AAA TGC TGG ACA					769
GTG TCA TCA AAA ATT ACA AGC ACT AGT TTC CTT GTG ATC TAT					811
GGC AAA GCC TCA GAG TGC ATG CAG GTG ATG TTT GGC ATT GAC					853
ATG AAG GAA GTG GAC CCC GCG GCC ACT CCT ACG TCC TTG TCA					895
CCT GCT TGG GCC TCT CCT ACA ATG GCC TGC TGG GTG ATG ATC					937
AGA GCA TGC CCG AGA CCG GCC TTC TGA					964
TTATGGTCTT GACCATGATC TTAATGGAGG GCCACTGTGC CCCTGAGGAG					1014
GCAATCTGGG AAGCGTTGAG TGTAATGGTG TATGATGGGA TGGAGCAGTT					1064
TCTTTGGGCA GCTGAGGAAG CTGCTCACCC AAGATTGGGT GCAGGAAAAC					1114
TACCTGCAAT ACCGCCAGGT GCCCAGCAGT GATCCCCCGT GCTACCAGTT					1164
CCTGTGGGGT CCAAGGGCCC TCATTGAAAC CAGCTATGTG AAAGTCCTGG					1214
AGTATGCAGC CAGGGTCAGT ACTAAAGAGA GCATTTCTTA CCCATCCCTG					1264
CATGAAGAGG CTTTGGGAGA GGAGGAAGAG GGAGTCTGAG CAGAAGTTGC					1314
AGCCAGGGCC AGTGGGGCAG ATTGGGGGAG GGCCTGGGCA GTGCACGTTT					1364
CACACATCCA CCACCTTCCC TGTCTGTGTA CATGAGGCCC ATTCTTCACT					1414
CTGTGTTTGA AGAGAGCAGT CAATGTTCTC AGTAGCGGGG AGTGTGTTGG					1464
GTGTGAGGGA ATACAAGGTG GACCATCTCT CAGTTCCTGT TCTCTTGGGC					1514
GATTTGGAGG TTTATCTTTG TTTCCCTTTG CAGTCGTTCA AATGTTCCCT					1564
TTAATGGATG GTGTAATGAA CTTCAACATT CATTTTCATGT ATGACAGTAG					1614
GCAGACTTAC TGTTTTTTAT ATAGTTAAAA GTAAGTGCAT TGTTTTTTAT					1664
TTATGTAAGA AAATCTATGT TATTTCTTGA ATTGGGACAA CATAACATAG					1714
CAGAGGATTA AGTACCTTTT ATAATGTGAA AGAACAAAGC GGTAATAATGG					1764
GTGAGATAAA GAAATAAAGA AATTAAATTG GCTGGGCACG GTGGCTCACG					1814
CCTGTAATCC CAGCACTTTA GGAGGCAGAG GCACGGGGAT CACGAGGTCA					1864
GGAGATCGAG ACCATTCTGG CTAACACAGT GAAACACCAT CTCTATTAAA					1914
AATACAAAAC TTAGCCGGGC GTGGTGGCGG GTG					1947

SUBSTITUTE SHEET

30

- (2) INFORMATION FOR SEQUENCE ID NO: 20:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 1810 base pairs
    - (B) TYPE: nucleic acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: genomic DNA
  - (ix) FEATURE:
    - (A) NAME/KEY: MAGE-8 gene
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```

GAGCTCCAGG AACCAGGCTG TGAGGTCTTG GTCTGAGGCA GTATCTTCAA      50
TCACAGAGCA TAAGAGGCCA AGGCAGTAGT AGCAGTCAAG CTGAGGTGGT      100
GTTTCCCCTG TATGTATACC AGAGGCCCTT CTGGCATCAG AACAGCAGGA      150
ACCCACAGT TCCTGGCCCT ACCAGCCCTT TTGTCAGTCC TGGAGCCTTG      200
GCCTTTGCCA GGAGGCTGCA CCCTGAGATG CCCTCTCAAT TTCTCCTTCA      250
GGTTCGAGCA GAACAGGCCA GCCAGGAGGT CAGGAGGCCA CAGAGAAGCA      300
CTGAAGAAGA CCTGTAAGTA GACCTTTGTT AGGGCATCCA GGGTGTAGTA      350
CCCAGCTGAG GCCTCTCACA CGCTTCCTCT CTCCCAGGC CTGTGGGTCT      400
CAATTGCCCA GCTCCGGCCC ACACTCTCCT GCTGCCCTGA CCTGAGTCAT      450
C                                                                451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA      493
GGC CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG      535
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC      577
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT      619
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT      661
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT      703
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC      745
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT      787
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA      829
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG      871
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC      913
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT      955
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC      997
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT     1039
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC     1081
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC     1123
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA                     1156
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAG     1206
AGTGGGTGCA GGAGAACTAC CTGGAGTACC GCCAGGCGCC CGGCAGTGAT     1256
CCTGTGCGCT ACGAGTTCCT GTGGGGTCCA AGGGCCCTTG CTGAAACCAG     1306
CTATGTGAAA GTCCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTCGCA     1356
TTTCCTACCC ATCCCTGCAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT     1406
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTTGTG GGAGGGCCTG     1456
GGCCAGTGCA CGTTCAGGG CCACATCCAC CACTTTCCTT GCTCTGTTAC     1506
ATGAGGCCCA TTCTTCACTC TGTGTTTGAA GAGAGCAGTC ACAGTTCTCA     1556
GTAGTGGGGA GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC     1606
AGTTCCTGTT CTATTGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTTGG     1656
AATTGTTCCA ATGTTCTTTC TAATGGATGG TGTAATGAAC TTCAACATTC     1706
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTATA TAGTTTAGGA     1756
GTAAGAGTCT TGCTTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA     1806
ATTC                                                                1810

```

SUBSTITUTE SHEET

91

## (2) INFORMATION FOR SEQUENCE ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1412 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-9 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TC TGAGACAG	TG TCCTCAGG	TC GCAGAGCA	GAG GAGACCC	AG GCAGTGTC	50
AG CAGTGAAG	GT GAAGTGTT	CACCCTGAAT	GT GCACCAAG	GG CCCCACCT	100
GG CCCCAGCAC	AC ATGGGACC	CC ATAGCACC	TG GCCCCATT	CC CCCTACTG	150
TC ACTCATAG	AG CCTTGATC	TCTGCAGGCT	AG CTGCACGC	TG AGTAGCCC	200
TCTCACTTCC	TCCCTCAGGT	TCTCGGGACA	GG CTAACCAG	GAG GACAGGA	250
GG CCCAAGAG	GG CCCCAGAGC	AG CACTGACG	AAG ACCTGTA	AG TCAGCCTT	300
TG TTAGAACC	TCCAAGGTTT	GG TTCTCAGC	TGA AGTCTCT	CAC ACACTCC	350
CT CTCTCCCC	AG GCCTGTGG	GT CTCCATCG	CC CAGCTCCT	GG CCACGCTC	400
CT GACTGCTG	CC CTGACCAG	AG TCATC			427
ATG TCT CTC	GAG CAG AGG	AGT CCG CAC	TGC AAG CCT	GAT GAA	469
GAC CTT GAA	GCC CAA GGA	GAG GAC TTG	GGC CTG ATG	GGT GCA	511
CAG GAA CCC	ACA GGC GAG	GAG GAG GAG	ACT ACC TCC	TCC TCT	553
GAC AGC AAG	GAG GAG GAG	GTG TCT GCT	GCT GGG TCA	TCA AGT	595
CCT CCC CAG	AGT CCT CAG	GGA GGC GCT	TCC TCC TCC	ATT TCC	637
GTC TAC TAC	ACT TTA TGG	AGC CAA TTC	GAT GAG GGC	TCC AGC	679
AGT CAA GAA	GAG GAA GAG	CCA AGC TCC	TCG GTC GAC	CCA GCT	721
CAG CTG GAG	TTC ATG TTC	CAA GAA GCA	CTG AAA TTG	AAG GTG	763
GCT GAG TTG	GTT CAT TTC	CTG CTC CAC	AAA TAT CGA	GTC AAG	805
GAG CCG GTC	ACA AAG GCA	GAA ATG CTG	GAG AGC GTC	ATC AAA	847
AAT TAC AAG	CGC TAC TTT	CCT GTG ATC	TTC GGC AAA	GCC TCC	889
GAG TTC ATG	CAG GTG ATC	TTT GGC ACT	GAT GTG AAG	GAG GTG	931
GAC CCC GCC	GGC CAC TCC	TAC ATC CTT	GTC ACT GCT	CTT GGC	973
CTC TCG TGC	GAT AGC ATG	CTG GGT GAT	GGT CAT AGC	ATG CCC	1015
AAG GCC GCC	CTC CTG ATC	ATT GTC CTG	GGT GTG ATC	CTA ACC	1057
AAA GAC AAC	TGC GCC CCT	GAA GAG GTT	ATC TGG GAA	GCG TTG	1099
AGT GTG ATG	GGG GTG TAT	GTT GGG AAG	GAG CAC ATG	TTC TAC	1141
GGG GAG CCC	AGG AAG CTG	CTC ACC CAA	GAT TGG GTG	CAG GAA	1183
AAC TAC CTG	GAG TAC CGG	CAG GTG CCC	GGC AGT GAT	CCT GCG	1225
CAC TAC GAG	TTC CTG TGG	GGT TCC AAG	GCC CAC GCT	GAA ACC	1267
AGC TAT GAG	AAG GTC ATA	AAT TAT TTG	GTC ATG CTC	AAT GCA	1309
AGA GAG CCC	ATC TGC TAC	CCA TCC CTT	TAT GAA GAG	GTT TTG	1351
GGA GAG GAG	CAA GAG GGA	GTC TGA			1375
GC ACCAGCCG	CAG CCGGGC	CAAAGTTTGT	GGGGTCA		1412

SUBSTITUTE SHEET

92

- (2) INFORMATION FOR SEQUENCE ID NO: 22:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 920 base pairs
    - (B) TYPE: nucleic acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: genomic DNA
  - (ix) FEATURE:
    - (A) NAME/KEY: MAGE-10 gene
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ACCTGCTCCA	GGACAAAGTG	GACCCCACTG	CATCAGCTCC	ACCTACCCTA	50
CTGTCAGTCC	TGGAGCCTTG	GCCTCTGCCG	GCTGCATCCT	GAGGAGCCAT	100
CTCTCACTTC	CTTCTTCAGG	TTCTCAGGGG	ACAGGGAGAG	CAAGAGGTCA	150
AGAGCTGTGG	GACACCACAG	AGCAGCACTG	AAGGAGAAGA	CCTGTAAGTT	200
GGCCTTTGTT	AGAACCTCCA	GGGTGTGGTT	CTCAGCTGTG	GCCACTTACA	250
CCCTCCCTCT	CTCCCCAGGC	CTGTGGGTCC	CCATCGCCCA	AGTCCTGCCC	300
ACACTCCCAC	CTGCTACCCT	GATCAGAGTC	ATC		333
ATG CCT CGA	GCT CCA AAG	CGT CAG CGC	TGC ATG CCT	GAA GAA	375
GAT CTT CAA	TCC CAA AGT	GAG ACA CAG	GGC CTC GAG	GGT GCA	417
CAG GCT CCC	CTG GCT GTG	GAG GAG GAT	GCT TCA TCA	TCC ACT	459
TCC ACC AGC	TCC TCT TTT	CCA TCC TCT	TTT CCC TCC	TCC TCC	501
TCT TCC TCC	TCC TCC TGC	TAT CCT CTA	ATA CCA AGC	ACC	543
CCA GAG GAG	GTT TCT GCT	GAT GAT GAG	ACA CCA AAT	CCT CCC	585
CAG AGT GCT	CAG ATA GCC	TGC TCC TCC	CCC TCG GTC	GTT GCT	627
TCC CTT CCA	TTA GAT CAA	TCT GAT GAG	GGC TCC AGC	AGC CAA	669
AAG GAG GAG	AGT CCA AGC	ACC CTA CAG	GTC CTG CCA	GAC AGT	711
GAG TCT TTA	CCC AGA AGT	GAG ATA GAT	GAA AAG GTG	ACT GAT	753
TTG GTG CAG	TTT CTG CTC	TTC AAG TAT	CAA ATG AAG	GAG CCG	795
ATC ACA AAG	GCA GAA ATA	CTG GAG AGT	GTC ATA AAA	AAT TAT	837
GAA GAC CAC	TTC CCT TTG	TTG TTT AGT	GAA GCC TCC	GAG TGC	879
ATG CTG CTG	GTC TTT GGC	ATT GAT GTA	AAG GAA GTG	GAT CC	920

SUBSTITUTE SHEET



93

## (2) INFORMATION FOR SEQUENCE ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1107 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: MAGE-11 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGAGAACAGG	CCAACCTGGA	GGACAGGAGT	CCCAGGAGAA	CCCAGAGGAT	50
CACTGGAGGA	GAACAAGTGT	AAGTAGGCCT	TTGTTAGATT	CTCCATGGTT	100
CATATCTCAT	CTGAGTCTGT	TCTCAGCTC	CCTCTCTCCC	CAGGCTGTGG	150
GGCCCCATCA	CCCAGATATT	TCCCACAGTT	CGGCCTGCTG	ACCTAACCAG	200
AGTCATCATG	CCTCTTGAGC	AAAGAAGTCA	GCACTGCAAG	CCTGAGGAAG	250
CCTTCAGGCC	CAAGAAGAAG	ACCTGGGCCT	GGTGGGTGCA	CAGGCTCTCC	300
AAGCTGAGGA	GCAGGAGGCT	GCCTTCTTCT	CCTCTACTCT	GAATGTGGGC	350
ACTCTAGAGG	AGTTGCCTGC	TGCTGAGTCA	CCAAGTCCTC	CCCAGAGTCC	400
TCAGGAAGAG	TCCTTCTCTC	CCACTGCCAT	GGATGCCATC	TTTGGGAGCC	450
TATCTGATGA	GGGCTCTGGC	AGCCAAGAAA	AGGAGGGGGC	AAGTACCTCG	500
CCTGACCTGA	TAGACCCTGA	GTCCTTTTCC	CAAGATATAC	TACATGACAA	550
GATAATTGAT	TTGGTTCATT	TATTCTCCGC	AAGTATCGAG	TCAAGGGGCT	600
GATCACAAAG	GCAGAA				616
ATG CTG GGG	AGT GTC ATC AAA	AAT TAT GAG	GAC TAC TTT	CCT	658
GAG ATA TTT	AGG GAA GCC TCT	GTA TGC ATG	CAA CTG CTC	TTT	700
GGC ATT GAT	GTG AAG GAA GTG	GAC CCC ACT	AGC CAC TCC	TAT	742
GTC CTT GTC	ACC TCC CTC AAC	CTC TCT TAT	GAT GGC ATA	CAG	784
TGT AAT GAG	CAG AGC ATG CCC	AAG TCT GGC	CTC CTG ATA	ATA	826
GTC CTG GGT	GTA ATC TTC ATG	GAG GGG AAC	TGC ATC CCT	GAA	868
GAG GTT ATG	TGG GAA GTC CTG	AGC ATT ATG	GGG GTG TAT	GCT	910
GGA AGG GAG	CAC TTC CTC TTT	GGG GAG CCC	AAG AGG CTC	CTT	952
ACC CAA AAT	TGG GTG CAG GAA	AAG TAC CTG	GTG TAC CGG	CAG	994
GTG CCC GGC	ACT GAT CCT GCA	TGC TAT GAG	TTC CTG TGG	GGT	1036
CCA AGG GCC	CAC GCT GAG ACC	AGC AAG ATG	AAA GTT CTT	GAG	1078
TAC ATA GCC	AAT GCC AAT GGG	AGG GAT CC			1107

SUBSTITUTE SHEET

## (2) INFORMATION FOR SEQUENCE ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2150 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: smage-I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TCTGTCTGCA	TATGCCTCCA	CTTGTGTGTA	GCAGTCTCAA	ATGGATCTCT	50
CTCTACAGAC	CTCTGTCTGT	GTCTGGCACC	CTAAGTGGCT	TTGCATGGGC	100
ACAGGTTTCT	GCCCCCTGCAT	GGAGCTTAAA	TAGATCTTTC	TCCACAGGCC	150
TATACCCCTG	CATTGTAAGT	TTAAGTGGCT	TTATGTGGAT	ACAGGTCTCT	200
GCCCTTGAT	GCAGGCCTAA	GTTTTTCTGT	CTGCTTAACC	CCTCCAAGTG	250
AAGCTAGTGA	AAGATCTAAC	CCACTTTTGG	AAGTCTGAAA	CTAGACTTTT	300
ATGCAGTGGC	CTAACAAGTT	TTAATTTCTT	CCACAGGGTT	TGCAGAAAAG	350
AGCTTGATCC	ACGAGTTCAG	AAGTCCTGGT	ATGTTCCCTAG	AAAG	394
ATG TTC TCC	TGG AAA GCT	TCA AAA GCC	AGG TCT CCA	TTA AGT	436
CCA AGG TAT	TCT CTA CCT	GGT AGT ACA	GAG GTA CTT	ACA GGT	478
TGT CAT TCT	TAT CCT TCC	AGA TTC CTG	TCT GCC AGC	TCT TTT	520
ACT TCA GCC	CTG AGC ACA	GTC AAC ATG	CCT AGG GGT	CAA AAG	565
AGT AAG ACC	CGC TCC CGT	GCA AAA CGA	CAG CAG TCA	CGC AGG	604
GAG GTT CCA	GTA GTT CAG	CCC ACT GCA	GAG GAA GCA	GGG TCT	646
TCT CCT GTT	GAC CAG AGT	GCT GGG TCC	AGC TTC CCT	GGT GGT	688
TCT GCT CCT	CAG GGT GTG	AAA ACC CCT	GGA TCT TTT	GGT GCA	730
GGT GTA TCC	TGC ACA GGC	TCT GGT ATA	GGT GGT AGA	AAT GCT	772
GCT GTC CTG	CCT GAT ACA	AAA AGT TCA	GAT GGC ACC	CAG GCA	814
GGG ACT TCC	ATT CAG CAC	ACA CTG AAA	GAT CCT ATC	ATG AGG	856
AAG GCT AGT	GTG CTG ATA	GAA TTC CTG	CTA GAT AAA	TTT AAG	898
ATG AAA GAA	GCA GTT ACA	AGG AGT GAA	ATG CTG GCA	GTA GTT	940
AAC AAG AAG	TAT AAG GAG	CAA TTC CCT	GAG ATC CTC	AGG AGA	982
ACT TCT GCA	CGC CTA GAA	TTA GTC TTT	GGT CTT GAG	TTG AAG	1024
GAA ATT GAT	CCC AGC ACT	CAT TCC TAT	TTG CTG GTA	GGC AAA	1066
CTG GGT CTT	TCC ACT GAG	GGA AGT TTG	AGT AGT AAC	TGG GGG	1108
TTG CCT AGG	ACA GGT CTC	CTA ATG TCT	GTC CTA GGT	GTG ATC	1150
TTC ATG AAG	GGT AAC CGT	GCC ACT GAG	CAA GAG GTC	TGG CAA	1192
TTT CTG CAT	GGA GTG GGG	GTA TAT GCT	GGG AAG AAG	CAC TTG	1234
ATC TTT GGC	GAG CCT GAG	GAG TTT ATA	AGA GAT GTA	GTG CGG	1276
GAA AAT TAC	CTG GAG TAC	CGC CAG GTA	CCT GGC AGT	GAT CCC	1314
CCA AGC TAT	GAG TTC CTG	TGG GGA CCC	AGA GCC CAT	GCT GAA	1360
ACA ACC AAG	ATG AAA GTC	CTG GAA GTT	TTA GCT AAA	GTC AAT	1402
GGC ACA GTC	CCT AGT GCC	TTC CCT AAT	CTC TAC CAG	TTG GCT	1444
CTT AGA GAT	CAG GCA GGA	GGG GTG CCA	AGA AGG AGA	GTT CAA	1486
GGC AAG GGT	GTT CAT TCC	AAG GCC CCA	TCC CAA AAG	TCC TCT	1528
AAC ATG TAG					1537
TTGAGTCTGT	TCTGTTGTGT	TTGAAAAACA	GTCAGGCTCC	TAATCAGTAG	1587
AGAGTTCATA	GCCTACCAGA	ACCAACATGC	ATCCATTCTT	GGCCTGTTAT	1637
ACATTAGTAG	AATGGAGGCT	ATTTTGTGTA	CTTTTCAAAT	GTTTGTTTAA	1687
CTAAACAGTG	CTTTTGGCCA	TGCTTCTTGT	TAAGTGCATA	AAGAGGTAAC	1737
TGTCACCTTGT	CAGATTAGGA	CTTGTTTTGT	TATTTGCAAC	AAACTGGAAA	1787

SUBSTITUTE SHEET

ACATTATTTT	GTTTTTACTA	AAACATTGTG	TAACATTGCA	TTGGAGAAGG	1837
GATTGTCATG	GCAATGTGAT	ATCATACAGT	GGTGAAACAA	CAGTGAAGTG	1887
GGAAAGTTTA	TATTGTTAAT	TTTGAAAATT	TTATGAGTGT	GATTGCTGTA	1937
TACTTTTTTC	TTTTTTGTAT	AATGCTAAGT	GAAATAAAGT	TGGATTTGAT	1987
GACTTTACTC	AAATTCATTA	GAAAGTAAAT	CGTAAAACTC	TATTACTTTA	2037
TTATTTTCTT	CAATTATGAA	TTAAGCATTG	GTTATCTGGA	AGTTTCTCCA	2087
GTAGCACAGG	ATCTAGTATG	AAATGTATCT	AGTATAGGCA	CTGACAGTGA	2137
GTTATCAGAG	TCT				2150

**SUBSTITUTE SHEET**

96

## (2) INFORMATION FOR SEQUENCE ID NO: 25:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2099 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

## (ix) FEATURE:

(A) NAME/KEY: smage-II

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACCTTATTGG	GTCTGTCTGC	ATATGCCTCC	ACTTGTGTGT	AGCAGTCTCA	50
AATGGATCTC	TCTCTACAGA	CCTCTGTCTG	TGTCTGGCAC	CCTAAGTGGC	100
TTTGCATGGG	CACAGGTTTC	TGCCCCTGCA	TGGAGCTTAA	ATAGATCTTT	150
CTCCACAGGC	CTATACCCCT	GCATTGTAA	TTTAAGTGGC	TTTATGTGGA	200
TACAGGTCTC	TGCCCTTGTA	TGCAGGCCA	AGTTTTTCTG	TCTGCTTAGC	250
CCCTCCAAGT	GAAGCTAGTG	AAAGATCTAA	CCCACTTTTG	GAAGTCTGAA	300
ACTAGACTTT	TATGCAGTGG	CCTAACAACT	TTTAATTTCT	TCCACAGGGT	350
TTGCAGAAAA	GAGCTTGATC	CACGAGTTTG	GAAGTCTGG	TATGTTCTTA	400
GAAAGATGTT	CTCCTGGAAA	GCTTCAAAAG	CCAGGTCTCC	ATTAAGTCCA	450
AGGTATTCTC	TACCTGGTAG	TACAGAGGTA	CTTACAGGTT	GTCATTCTTA	500
TCTTTCCAGA	TTCCTGTCTG	CCAGCTCTTT	TACTTCAGCC	CTGAGCACAG	550
TCAACATGCC	TAGGGGTCAA	AAGAGTAAGA	CCCGCTCCCG	TGCAAAACGA	600
CAGCAGTCAC	GCAGGGAGGT	TCCAGTAGTT	CAGCCCACTG	CAGAGGAAGC	650
AGGGTCTTCT	CCTGTTGACC	AGAGTGCTGG	GTCCAGCTTC	CCTGGTGGTT	700
CTGCTCCTCA	GGGTGTGAAA	ACCCCTGGAT	CTTTTGGTGC	AGGTGTATCC	750
TGCACAGGCT	CTGGTATAGG	TGGTAGAAAT	GCTGCTGTCC	TGCCTGATAC	800
AAAAAGTTCA	GATGGCACCC	AGGCAGGGAC	TTCCATTTCAG	CACACACTGA	850
AAGATCCTAT	CATGAGGAAG	GCTAGTGTGC	TGATAGAATT	CCTGCTAGAT	900
AAGTTTAAGA	TGAAAGAAGC	AGTTACAAGG	AGTGAAATGC	TGGCAGTAGT	950
TAACAAGAAG	TATAAGGAGC	AATTCCCTGA	GATCCTCAGG	AGAACTTCTG	1000
CACGCCTAGA	ATTAGTCTTT	GGTCTTGAGT	TGAAGGAAAT	TGATCCCAGC	1050
ACTCATTCTT	ATTTGCTGGT	AGGCAAACCTG	GGTCTTTCCA	CTGAGGGAAG	1100
TTTGAGTAGT	AACTGGGGGT	TGCCTAGGAC	AGGTCTCCTA	ATGTCTGTCC	1150
TAGGTGTGAT	CTTCATGAAG	GGTAACCGTG	CCACTGAGCA	AGAGGTCTGG	1200
CAATTTCTGC	ATGGAGTGGG	GGTATATGCT	GGGAAGAAGC	ACTTGATCTT	1250
TGGCGAGCCT	GAGGAGTTTA	TAAGAGATGT	AGTGCGGGAA	AATTACCTGG	1300
AGTACCGCCA	GGTACCTGGC	AGTGATCCCC	CAAGCTATGA	GTTCTGTGG	1350
GGACCCAGAG	CCCATGCTGA	AACAACCAAG	ATGAAAGTCC	TGGAAGTTTT	1400
AGCTAAAGTC	AATGGCACAG	TCCCTAGTGC	CTTCCCTAAT	CTCTACCAGT	1450
TGGCTCTTAG	AGATCAGGCA	GGAGGGGTGC	CAAGAAGGAG	AGTTCAAGGC	1500
AAGGGTGTTT	ATTCCAAGGC	CCCATCCCAA	AAGTCCTCTA	ACATGTAGTT	1550
GAGTCTGTTT	TGTTGTGTTT	GAAAAACAGT	CAGGCTCCTA	ATCAGTAGAG	1600
AGTTCATAGC	CTACCAGAAC	CAACATGCAT	CCATTCTTGG	CCTGTTATAC	1650
ATTAGTAGAA	TGGAGGCTAT	TTTTGTACT	TTTCAAATGT	TTGTTAACT	1700
AAACAGTGCT	TTTTGCCATG	CTTCTTGTTA	ACTGCATAAA	GAGGTAAGTG	1750
TCACTTGTC	GATTAGGACT	TGTTTTGTGA	TTTGCAACAA	ACTGGAACAA	1800
ATTATTTTGT	TTTTACTAAA	ACATTGTGTA	ACATTGCATT	GGAGAAGGGA	1850
TTGTCATGGC	AATGTGATAT	CATACAGTGG	TGAAACAACA	GTGAAGTGGG	1900
AAAGTTTATA	TTGTTAGTTT	TGAAAATTTT	ATGAGTGTGA	TTGCTGTATA	1950
CTTTTTTCTT	TTTTGTATAA	TGCTAAGTGA	AATAAAGTTG	GATTTGTATGA	2000
CTTTACTCAA	ATTCATTAGA	AAGTAAATCA	TAAACTCTA	TTACTTTATT	2050
ATTTTCTTCA	ATTATTAATT	AAGCATTGGT	TATCTGGAAG	TTTCTCCAG	2099

SUBSTITUTE SHEET

97

- (2) INFORMATION FOR SEQUENCE ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9 amino acids
    - (B) TYPE: amino acids
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: protein
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Ala Asp Pro Thr Gly His Ser Tyr

5

**SUBSTITUTE SHEET**

**Claims:**

1. Isolated nucleic acid molecule which codes for a tumor rejection antigen precursor or is complementary to a nucleic acid molecule which codes for a tumor rejection antigen precursor.
2. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a tumor rejection antigen precursor.
3. Isolated nucleic acid molecule of claim 1, wherein said molecule codes for a human tumor rejection antigen precursor.
4. The isolated nucleic acid molecule of claim 1, wherein said molecule is complementary to a nucleic acid molecule which codes for tumor rejection antigen precursor.
5. The isolated nucleic acid molecule of claim 1, wherein said molecule is DNA.
6. The isolated nucleic acid molecule of claim 1, wherein said molecule is RNA.
7. The isolated nucleic acid molecule of claim 1, wherein said molecule is a gene.

8. The isolated nucleic acid molecule of claim 5, wherein said DNA is genomic DNA.
9. The isolated nucleic acid molecule of claim 5, wherein said DNA is cDNA.
10. The isolated nucleic acid molecule of claim 6, wherein said RNA is mRNA.
11. The isolated nucleic acid molecule of claim 4, wherein said molecule hybridizes to isolated nucleic acid which codes for tumor rejection antigen precursor under stringent conditions.
12. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a MAGE antigen precursor or is complementary to a molecule which codes for a MAGE antigen precursor.
13. The isolated nucleic acid molecule of claim 12, wherein said MAGE antigen precursor is selected from the group consisting of mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.
14. The isolated nucleic acid molecule of claim 12, wherein said molecule codes for a MAGE antigen precursor.

15. The isolated nucleic acid molecule of claim 12, wherein said molecule is complementary to a molecule which codes for a MAGE antigen precursor.
16. The isolated nucleic acid molecule of claim 12, wherein said molecule is DNA.
17. The isolated nucleic acid molecule of claim 12, wherein said molecule is RNA.
18. The isolated nucleic acid molecule of claim 12, wherein said molecule is a gene.
19. The isolated nucleic acid molecule of claim 16, wherein said DNA is genomic DNA.
20. The isolated nucleic acid molecule of claim 16, wherein said DNA is cDNA.
21. The isolated nucleic acid molecule of claim 17, wherein said RNA is mRNA.
22. The isolated nucleic acid molecule of claim 12, comprising a nucleotide sequence set forth in figure 9.



23. The isolated nucleic acid molecule of claim 15, wherein said molecule hybridizes to a molecule which codes for a MAGE antigen precursor under stringent conditions.
24. Isolated nucleic acid molecule of claim 1, coding for a tumor rejection antigen precursor for mastocytoma.
25. Isolated nucleic acid molecule of claim 1, coding for tumor rejection antigen precursor P1A.
26. Isolated nucleic acid molecule of claim 1, having the nucleotide sequence of figure 5.
27. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 2.
28. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 12.
29. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 22.
30. Biologically pure culture of a cell line of claim 27, selected from the group consisting of P1A.T2 and P1A.TC3.1.

31. Biologically pure culture of a highly transfectable cell line derived from a parent cell line which expresses at least one P815 tumor antigen, wherein said highly transfectable cell line does not express any of P815 tumor antigens A, B and C.
32. Biologically pure cell line of claim 31, comprising cell line PO.HTR.
33. Biologically pure culture of a cell line of claim 27, wherein said tumor rejection antigen precursor is a human tumor antigen precursor.
34. Biologically pure culture of a cell line of claim 33, wherein said human tumor antigen precursor is found in melanoma cells.

163

35. Biologically pure cell line of claim 34, said tumor rejection antigen precursor is mage-1 and said isolated DNA has nucleic acid sequence:

	1	10	20	30	40	50	60
1	GGATCCAGGC	CCTGCCAGGA	AAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGGTCATCC	60
61	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGGCCACCC	TCCTGTAGC	ACTGAGAGGC	120
121	CAGGGCTGTG	CTTGGCGTCT	GCACCCCTGAG	GGCCCGTGG	TTCTCTTCC	TGGAGCTCCA	180
181	GGAACAGGC	AGTGAAGCCT	TGCTCTGAG	CATATCCCTC	AGGTCAAGA	GCAGAGGATG	240
241	CACAGGCTGT	GCCAGCAGTG	AATGTTTGGC	CTGAATGCAC	ACCAAGGCTC	CCACCTGCCA	300
301	CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	CCTGTAGAA	360
361	CGACCTCTGC	TGGCCGGCTG	TAACCTGAGT	ACCTCTCAC	TTCTCTCTC	AGGTCTCTAG	420
421	GGGACAGGTC	AACCCAGAGG	ACAGGATTC	CTGGAGGCA	CAGAGGAGCA	CCAAGGAGAA	480
481	GATCTGTAA	TAGGCTCTTG	TTAGAGTCTC	CAAGGTTGAG	TTCTCAGCTG	AGGCTCTCTA	540
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGCT	CTTCAATTGC	CAGCTCCTGC	CCAACCTCC	600
601	GCCTGCTGCC	CTGACGAGAG	TCAATCATGTC	TCCTGAGCAG	AGGAGTCTGC	ACTGCAAGCC	660
661	TGAGGAAGCC	CTTGAGGGCC	AACAAGAGGC	CTTGGGCTGG	TGTGTGTGCA	GGCTGCCACC	720
721	TCCTCTCTCT	CTCTCTCTGG	CCTGGGCACC	CTGGAGGAGG	TGCTCACTGC	TGGGTCAACA	780
781	GATCCTCCCC	AGAGTCTCTA	GGGAGCCTCC	GCCTTCCCA	CTACCATCAA	CTTCACTCGA	840
841	CAGAGGCAAC	CCAGTGAGGG	TTCCAGCAGC	CGTGAAAGAG	AGGTGCAAG	CACCTCTTGT	900
901	ATCCTGGAGT	CCTTGTCTCC	AGCAATTAATC	ACTAAGAAAG	TGGTGAATTT	GTTTGAATTT	960
961	CTGCTCCTCA	AAATATCGAGC	CAGGGAACCA	GTCAACAAAG	CAGAAATGCT	GGAGAGTGTC	1020
1021	ATCAAAATTT	ACAAGCACTG	TTTTCTGTAG	ATCTTGGCA	AAGCTCTGTA	GTCTTGTGAG	1080
1081	CTGGTCTTTG	GCACTGACGT	GAAGGAAGCA	GACCCCAACG	GCACTCTCTA	TGTCTCTCTC	1140
1141	ACCTGCCCTAG	GTCTCTCTTA	TGATGGCCTG	CTGGGTGATA	ATCAGATCAT	GCCCAAGACA	1200
1201	GGCTTCTGTA	TAAATGTCTT	GTTCAATGAT	GCAATGGAGG	GCGGCCCTGC	TCCTGAGGAG	1260
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCTAT	1320
1321	GGGGAGGCCA	GGAAGCTGCT	CACCCAAAT	TTGGTGCAGG	AAAGTACCT	GGAGTACGGC	1380
1381	AGGTGCCGGA	CAGTGAATCC	GCACGCTATG	AGTTCTCTGT	GGGTCCAAAG	GGCCTCTCTG	1440
1441	AAACCAAGCTA	TGTGAAGATC	CTTGAATATG	TGATCAAGGT	CAGTGCAGGA	GTTCCTCTTT	1500
1501	TCTTCCCATC	CCTGCCGTGA	GCAGCTTTGA	GAGAGTGAAG	AGAGGGAGTC	TGAGCAATGAG	1560
1561	TTGCAAGCCA	GCCCAAGTGG	AGGGGGAATG	GGCCAGTGCA	CCTTCCAGGG	CCCGCTCCAG	1620
1621	CAGCTTCCCC	TGCTCTCTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGTCACT	1680
1681	GTCTCTCACTA	GTAGGTTTCT	GTTCATTTGG	GTGACTTGGG	GATTTATCTT	TGTTCTCTTT	1740
1741	TGGAAATGTT	CAAAATGTTT	TTTTTAAGGG	ATGTTTGAAT	GAACTTCAGC	ATCCAAATTT	1800
1801	ATGAATGACA	GCACTCACAC	AGTTCTGTGT	ATATAGTTTA	AGGTAAAGAG	TCTTGTGTTT	1860
1861	TAATTCAGAT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAATTAACAGC	AGTGGAAATA	1920
1921	GTACTTAGAA	ATGTGAAGAA	TGAGCAGTAA	AAATAGATGAG	ATAAAGAACT	AAAGAAATTA	1980
1981	AGAGATAGTC	AATTCTTGGC	TTATACCTCA	GTCTATTTCTG	TAAATTTTCT	AAAGATATAT	2040
2041	GCATACCTGG	ATTTCTTGGG	CTTCTTTGAG	AAATGAAGAG	AAATTAATTC	TGAATAAAGA	2100
2101	ATCTTCTCTG	TTCACTGACT	CTTTTCTTCT	CCATGCACTG	AGCATCTGCT	TTTTGGAAGG	2160
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGTTCTG	2220
2221	AGAGTCTAGG	AGCTGCACTC	ACCTAATCGA	GGTGGCAAGA	TGTCTCTTAA	AGATGTAGGG	2280
2281	AAAGTGAAGA	GAGGGGTGAG	CGTGTGGGGC	TCCGGGTGAG	AGTGTGGAG	TGTCAATGCC	2340
2341	CTGAGCTGGG	GCAATTTGGG	CTTTGGGAAA	CTGCAATTC	TTCTGGGGGA	CTGATTGTA	2400
2401	ATGATCTTGG	GTGATTC					2418

36. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence coding for a cytokine.
37. The biologically pure culture of claim 36, wherein said cell line is further transfected by a nucleic acid sequence coding for an HLA molecule.
38. The biologically pure culture of claim 36, wherein said cytokine is an interleukin.
39. The biologically pure culture of claim 38, wherein said interleukin is IL-2.
40. The biologically pure culture of claim 38, wherein said interleukin is IL-4.
41. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence which codes for an MHC molecule or an HLA molecule.
42. The biologically pure culture of claim 27, wherein said cell line expresses an MHC or HLA molecule which presents a tumor rejection antigen derived from a tumor rejection antigen precursor (TRAP), wherein said TRAP is coded for by a nucleic acid sequence transfected into said cell line.

43. The biologically pure culture of claim 27, wherein said culture is non-proliferative.
44. The biologically pure culture of claim 27, wherein said cell line is a fibroblast cell line.
45. Transfected bacteria containing the nucleic acid sequence of claim 2.
46. Mutated virus containing the nucleic acid sequence of claim 2.
47. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 2 operably linked to a promoter.
48. Expression vector useful in transfecting a cell comprising a nucleic acid sequence coding for a tumor rejection antigen operably linked to a promoter.
49. Expression vector of claim 47, wherein said promoter is a strong promoter.
50. Expression vector of claim 47, wherein said promoter is a differential promoter.

51. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 7 operably linked to a promoter.
52. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 13 operably linked to a promoter.
53. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 14 operably linked to a promoter.
54. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 18 operably linked to a promoter.
55. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 22 operably linked to a promoter.
56. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for an MHC or HLA.
57. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for a cytokine.
58. The expression vector of claim 57, wherein said cytokine is an interleukin.

59. The expression vector of claim 58, wherein said interleukin is IL-2.
60. The expression vector of claim 58, wherein said interleukin is IL-4.
61. The expression vector of claim 47, further comprising a bacterial or viral genome or portion thereof.
62. The expression vector of claim 61, wherein said viral genome vaccinia virus DNA and said bacterial genome or portion thereof in BCG DNA.
63. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for a tumor rejection antigen precursor, and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor, and (b) a vector containing a nucleic acid sequence which codes for an interleukin.
64. Isolated tumor rejection antigen precursor.
65. Isolated human tumor rejection antigen precursor.

66. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is mage-1.
67. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is a precursor for antigen F.
68. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 2.
69. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 12.
70. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 13.
71. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 22.
72. Isolated tumor rejection antigen.
73. Isolated human tumor rejection antigen.
74. Isolated tumor rejection antigen of claim 72 having amino acid sequence of SEQ ID NO: 4.
75. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen E.



76. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen F.
77. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a tumor rejection antigen precursor which provokes an immune response when administered to a subject.
78. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a peptide fragment derived from a tumor rejection antigen precursor, wherein said fragment is larger than the tumor rejection antigen derived from said tumor rejection antigen precursor and smaller than said tumor rejection antigen precursor and which provokes an immune response when administered to a subject.
79. Vaccine of claim 77, wherein said TRAP is a human TRAP.
80. Vaccine of claim 77 wherein said precursor is mage-1.
81. Vaccine of claim 79, wherein said precursor is antigen F precursor.

82. Vaccine useful in treating a patient with a cancer comprising a tumor rejection antigen of claim 72 which provokes an immune response when administered to a subject.
83. Vaccine of claim 82, wherein said tumor rejection antigen has amino acid sequence of SEQ ID NO: 4.
84. The vaccine of claim 81, wherein said tumor rejection antigen is antigen E.
85. The vaccine of claim 81, wherein said tumor rejection antigen is antigen F.
86. The vaccine of claim 77, wherein said tumor rejection antigen precursor is the expression product of an expression vector containing a viral genome or portion thereof.
87. Vaccine useful in treating a patient with a cancer comprising the transfected bacterial of claim 45 and a pharmaceutically acceptable adjuvant.
88. Vaccine useful in treating a cancerous condition comprising the mutated virus of claim 46, and a pharmacologically acceptable adjuvant.

89. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a complex of a tumor rejection antigen and an HLA molecule.
90. Isolated peptide useful in treating a subject afflicted with a cancerous condition, said peptide having the amino acid of SEQ ID NO: 26.
91. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 27 and a pharmacologically acceptable adjuvant.
92. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 37 and a pharmacologically acceptable adjuvant.
93. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen precursor specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
94. Composition of matter of claim 93, wherein said cell line is a human cell line.

95. Composition of matter of claim 93, wherein said pharmaceutically acceptable carrier is a liposome.
96. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
97. Composition of matter of claim 96, wherein said cell line is a human cell line.
98. Composition of matter of claim 96, wherein said pharmaceutically acceptable carrier is a liposome.
99. Composition of matter useful in treating a cancerous condition, comprising (i) a tumor rejection antigen or tumor rejection antigen precursor, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.
100. Composition of matter of claim 99, wherein said pharmaceutically acceptable carrier is a liposome.
101. Antibody which specifically binds to a tumor rejection antigen precursor.

102. Antibody of claim 101, wherein said antibody is a monoclonal antibody.
103. Antibody of claim 101, wherein said tumor rejection antigen precursor is mage-1.
104. Antibody of claim 103, wherein said antibody is a monoclonal antibody.
105. Antibody of claim 101, wherein said tumor rejection antigen precursor is antigen F precursor.
106. Antibody of claim 105, wherein said antibody is a monoclonal antibody.
107. Antibody of claim 101, wherein said tumor rejection antigen precursor is a MAGE precursor.
108. Antibody of claim 107, wherein said antibody is a monoclonal antibody.
109. Antibody of claim 107, wherein said MAGE precursor is mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.
110. Antibody of claim 109, wherein said antibody is a monoclonal antibody.

111. Antibody which specifically binds to a tumor rejection antigen.
112. Antibody of claim 111, wherein said antibody is a monoclonal antibody.
113. Antibody of claim 111, wherein said tumor rejection antigen is that set forth in SEQ ID NO: 4.
114. Antibody of claim 113, wherein said antibody is a monoclonal antibody.
115. Antibody of claim 111, wherein said tumor rejection antigen is antigen E.
116. Antibody of claim 115, wherein said antibody is a monoclonal antibody.
117. Antibody of claim 111, wherein said tumor rejection antigen is antigen F.
118. Antibody of claim 117, wherein said antibody is a monoclonal antibody.
119. Antibody which specifically binds to a complex of (i) tumor rejection antigen and (ii) HLA molecule, but does not bind to (i) or (ii) alone.

120. The antibody of claim 119, wherein said antibody is a monoclonal antibody.
121. Method for diagnosing a cancerous condition in a subject, comprising contacting a lymphocyte containing sample of said subject to a cell line transfected with a DNA sequence coding for a tumor rejection antigen precursor expressed by cells associated with said cancerous condition, and determining lysis of said transfected cell line by a cytotoxic T cell line specific for a tumor rejection antigen derived from said tumor rejection antigen precursor, said lysis being indicative of said cancerous condition.
122. Method of claim 121, wherein said tumor rejection antigen precursor is a MAGE antigen.
123. Method for determining regression, progression or onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) tumor rejection antigen precursor, (ii) tumor rejection antigen and (iii) cytolytic T cells specific for a tumor rejection antigen associated with said cancerous condition, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

124. Method of claim 123, wherein said sample is a body fluid.
125. Method of claim 123, wherein said sample is a tissue.
126. Method of claim 123, comprising contacting said sample with an antibody which specifically binds with said tumor rejection antigen or tumor rejection antigen precursor.
127. Method of claim 126, wherein said antibody is labelled with a radioactive label or an enzyme.
128. Method of claim 126, wherein said antibody is a monoclonal antibody.
129. Method of claim 123, comprising amplifying RNA which codes for said tumor rejection antigen precursor.
130. Method of claim 129, wherein said amplifying comprises carrying out polymerase chain reaction.
131. Method of claim 123, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said tumor rejection antigen precursor.
132. Method of claim 123, comprising assaying said sample for shed tumor rejection antigen.



133. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for a cytolytic T cell specific for a tumor rejection antigen, presence of said cytolytic T cell being indicative of said cancerous condition.
134. Method for treating a subject afflicted with a cancerous condition, comprising:
- (i) removing a lymphocyte containing sample from said subject,
  - (ii) contacting the lymphocyte containing sample to a cell line transfected with a gene coding for and expressing a gene for a tumor rejection antigen precursor expressed by cancer cells associated with said conditions, under conditions favoring production of cytotoxic T cells against a tumor rejection antigen derived from said tumor rejection antigen precursor, and
  - (iii) introducing said cytotoxic T cells to said subject in an amount sufficient to lyse said cells.
135. Method for treating a subject afflicted with a cancerous condition, comprising:
- (i) identifying a MAGE gene expressed by cancer cells associated with said condition;
  - (ii) identifying an HLA molecule which presents a portion of an expression product of said MAGE gene;

(iii) transfecting a host cell having the same HLA molecule as identified in (ii) with said MAGE gene;

(iv) culturing said transfected cells to express said MAGE-gene, and;

(v) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

136. Method of claim 135, wherein said immune response comprises a B-cell response.

137. Method of claim 135, wherein said immune response is a T-cell response.

138. Method of claim 136, wherein said B cell response comprises production of antibodies specific to said tumor rejection antigen or tumor rejection antigen precursor.

139. Method of claim 137, wherein said T-cell response comprises generation of cytolytic T-cells specific for cells presenting said tumor rejection antigen.

140. Method of claim 139, further comprising treating said cells to render them non-proliferative.

141. Method for treating a subject with a cancerous condition, comprising:

(i) identifying a MAGE gene expressed by said tumor;

(ii) transfecting a host cell having the same HLA type as said patient with said MAGE gene;

(iii) culturing said transfected cells to express said MAGE gene, and;

(iv) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

142. Method of claim 141, further comprising treating said cells to render them non proliferative.

143. Method for treating a subject with a cancerous condition, comprising administering to said subject an amount of a cell transfected with (i) a nucleic acid sequence which codes for a tumor rejection antigen precursor (TRAP) and (ii) a nucleic acid sequence which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said TRAP, wherein said tumor rejection antigen is presented by cells associated with said cancerous condition, sufficient to alleviate said cancerous condition.

144. Method of claim 143, further comprising treating said cell to render it non-proliferative.

145. Method for preparing a biological material useful in treating a subject afflicted with a cancerous condition, comprising:

(i) transfecting a host cell with a nucleic acid molecule which codes for or expresses a tumor rejection antigen precursor;

(ii) transfecting said host cell with a nucleic acid molecule which codes for an HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor on a cell surface, and;

(iii) treating said host cells under conditions favoring expression of said nucleic acid molecules, and presentation of said tumor rejection antigen by said human leukocyte antigen.

146. Method of claim 145, further comprising treating said host cells to render them non proliferative following presentation of said tumor rejection antigen.

147. Method of claim 146, further comprising transfecting said host cell with a nucleic acid molecule which codes for or expresses a cytokine.

148. Method of claim 146, wherein said cytokine is an interleukin.

149. Method of claim 146, wherein said human leukocyte antigen is HLA-A1.
150. Method of claim 148, wherein said interleukin is IL-2.
151. Method of claim 146, wherein said interleukin is IL-4.
152. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an amount of a reagent consisting essentially of non-proliferative cell having expressed on its surface a tumor rejection antigen characteristic of cancerous cells in an amount sufficient to elicit an immune response thereto.
153. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a tumor rejection antigen expressed on a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.
154. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a

tumor rejection antigen precursor expressed by a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.

155. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject a biological sample prepared in accordance with claim 142 in an amount sufficient to alleviate said cancerous condition.
156. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 77 in an amount sufficient to prevent onset of said cancerous condition in said subject.
157. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 78 in an amount sufficient to prevent onset of said cancerous condition in said subject.
158. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 82 in an amount sufficient to prevent onset of said cancerous condition in said subject.

159. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 86 in an amount sufficient to prevent onset of said cancerous condition in said subject.
160. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 87 in an amount sufficient to prevent onset of said cancerous condition in said subject.
161. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 88 in an amount sufficient to prevent onset of said cancerous condition in said subject.
162. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.
163. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.

164. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 90 in an amount sufficient to prevent onset of said cancerous condition in said subject.

165. Method for treating a subject afflicted with a cancerous condition, comprising:

- (i) identifying cells from said subject which express a tumor rejection antigen precursor and present a tumor rejection antigen derived from said precursor on their surface;

- (ii) isolating a sample of said cells;

- (iii) cultivating said cell, and;

- (iv) introducing said cells to said subject in an amount sufficient to provoke an immune response against said cells.

166. Method of claim 165, further comprising rendering said cells non proliferative, prior to introducing them to said subject.

167. Method for identifying a cytotoxic T cell useful in treating a subject afflicted with a cancerous condition, comprising:

- (i) identifying a tumor rejection antigen presented by cells associated with said cancerous condition derived from a tumor rejection antigen



precursor expressed by said cells, prior to introducing them to said subject;

(ii) contacting a cell presenting said antigen to a cytotoxic T cell, and;

(iii) measuring a parameter selected from the group consisting of (i) proliferation of said cytotoxic T cell and (ii) release of a cytotoxic T cell produced factor, wherein increase in said parameter is indicative of said cancerous condition.

168. Method of claim 167, wherein said factor is tumor necrosis factor.

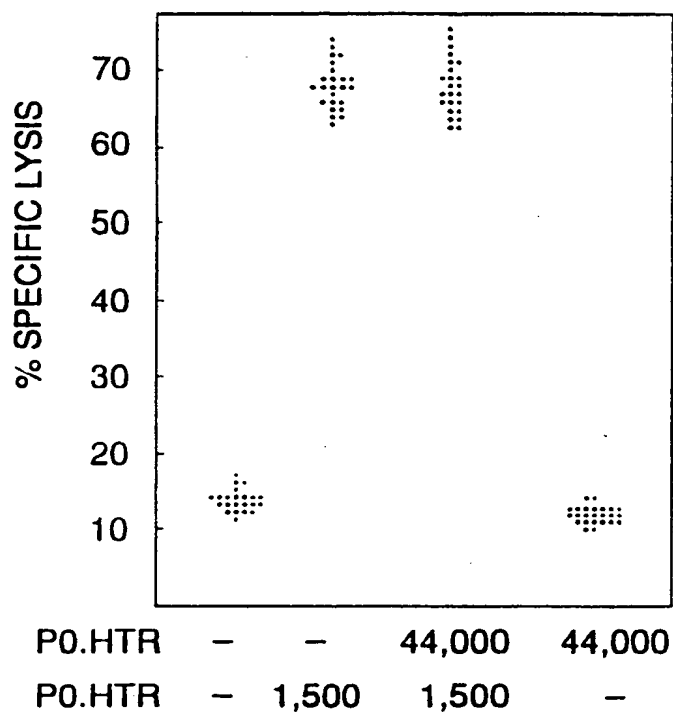
169. Method for following progress of a therapeutic regime designed to alleviate a cancerous condition, comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) tumor rejection antigen, (ii) a cytolytic T cell specific for cells presenting said tumor rejection antigen, and (iii) an antibody which specifically binds to said tumor rejection antigen at a first time period;

(b) assaying level of the parameter selected in (a) at a second period of time and comparing it to the level determined in (a) as a determination of effect of said therapeutic regime.

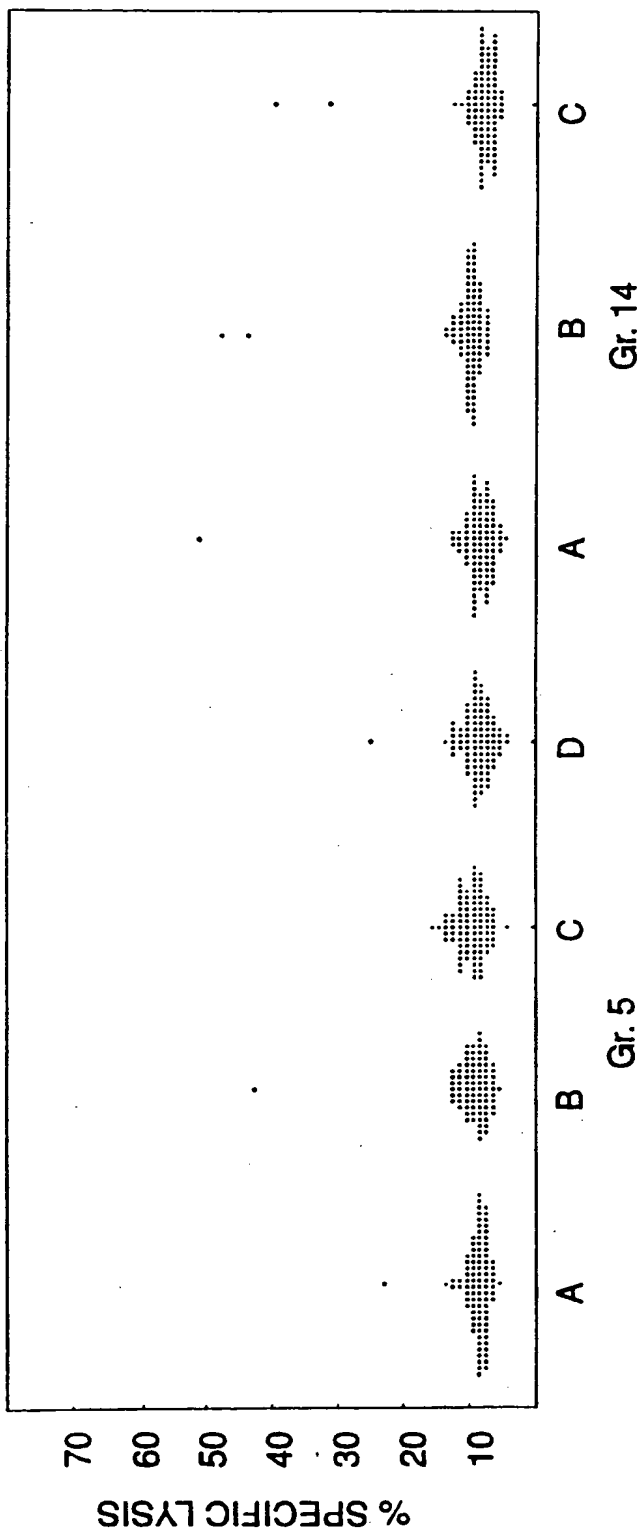
170. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for expression of a TRAP molecule, and comparing levels of expression to a normal level, wherein variance there between is indicative of a cancerous condition.
171. Method of claim 164, comprising measuring expression via polymerase chain reaction.
172. Method of claim 123, comprising intradermally administering an amount of a tumor rejection antigen sufficient to generate a delayed type response in a subject.

1/13

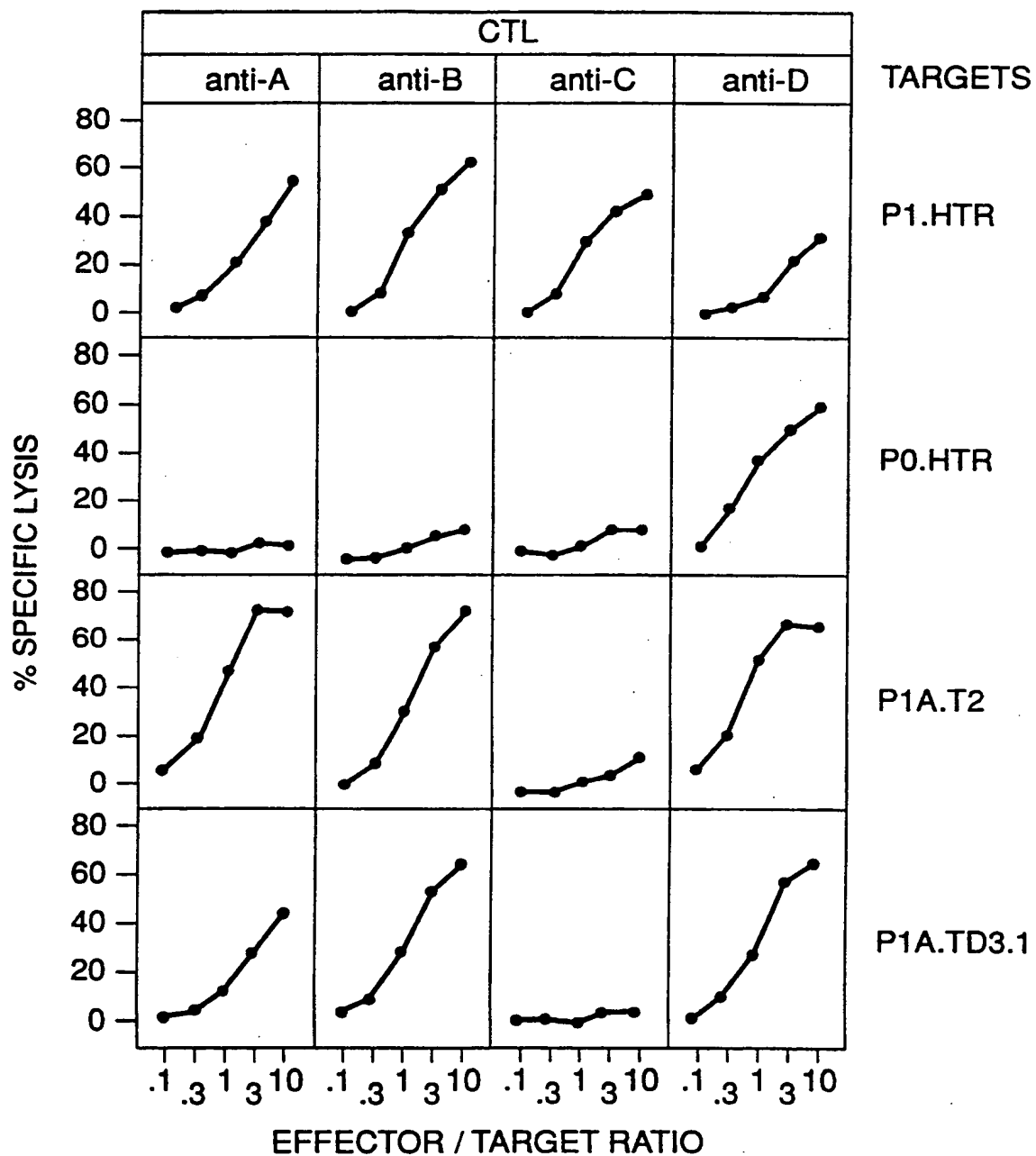
**FIG. 1A**

2/13

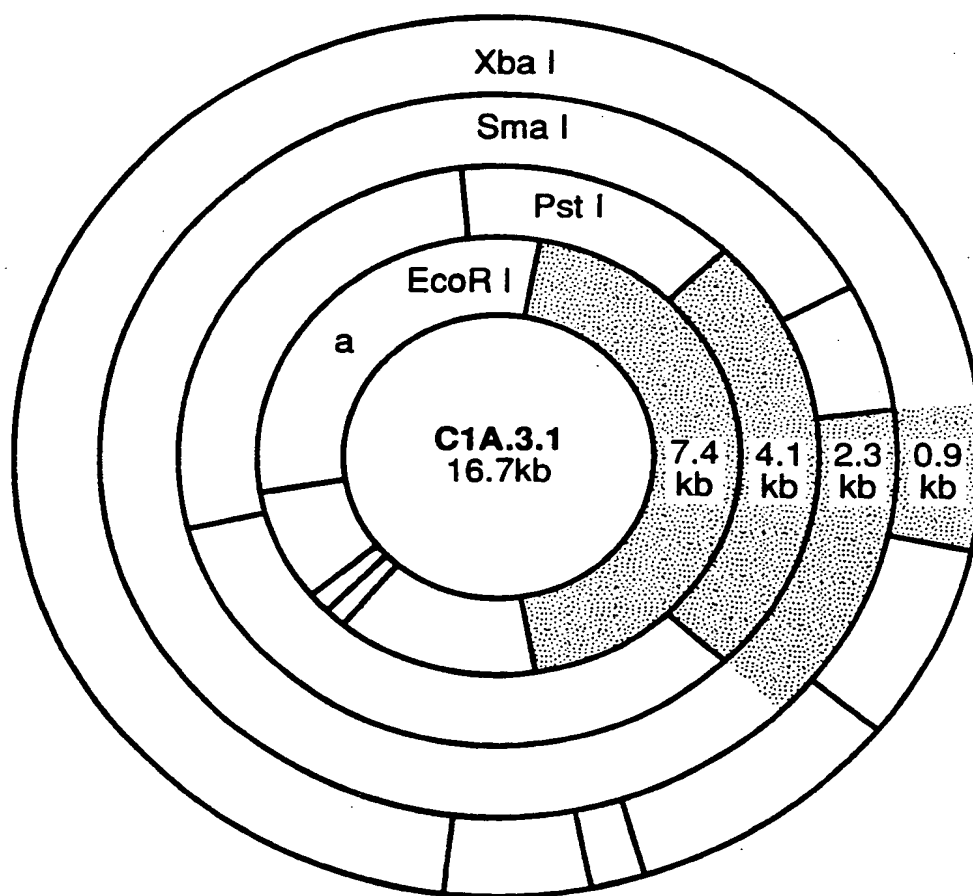
FIG. 1B



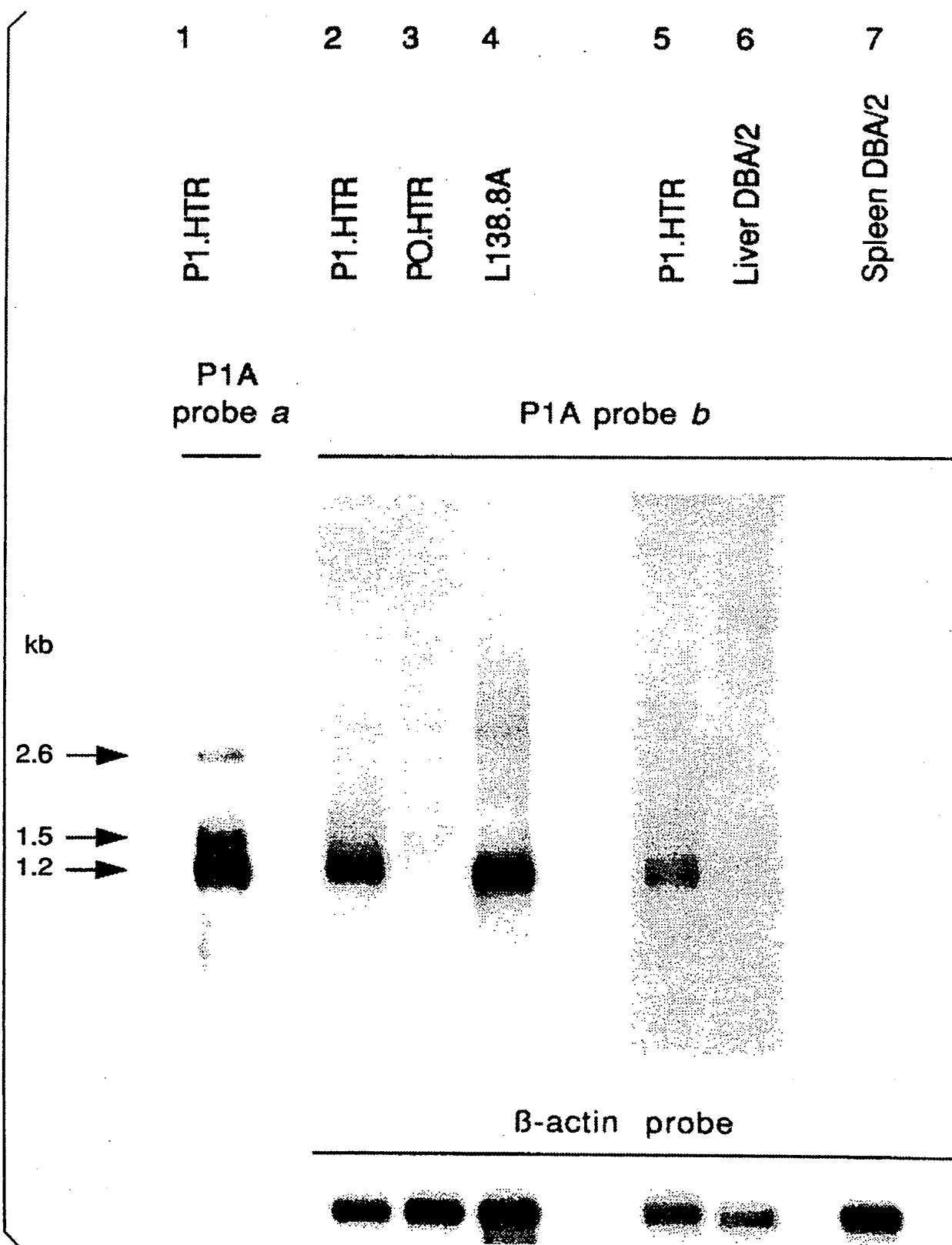
3/13

**FIG. 2**

4/13

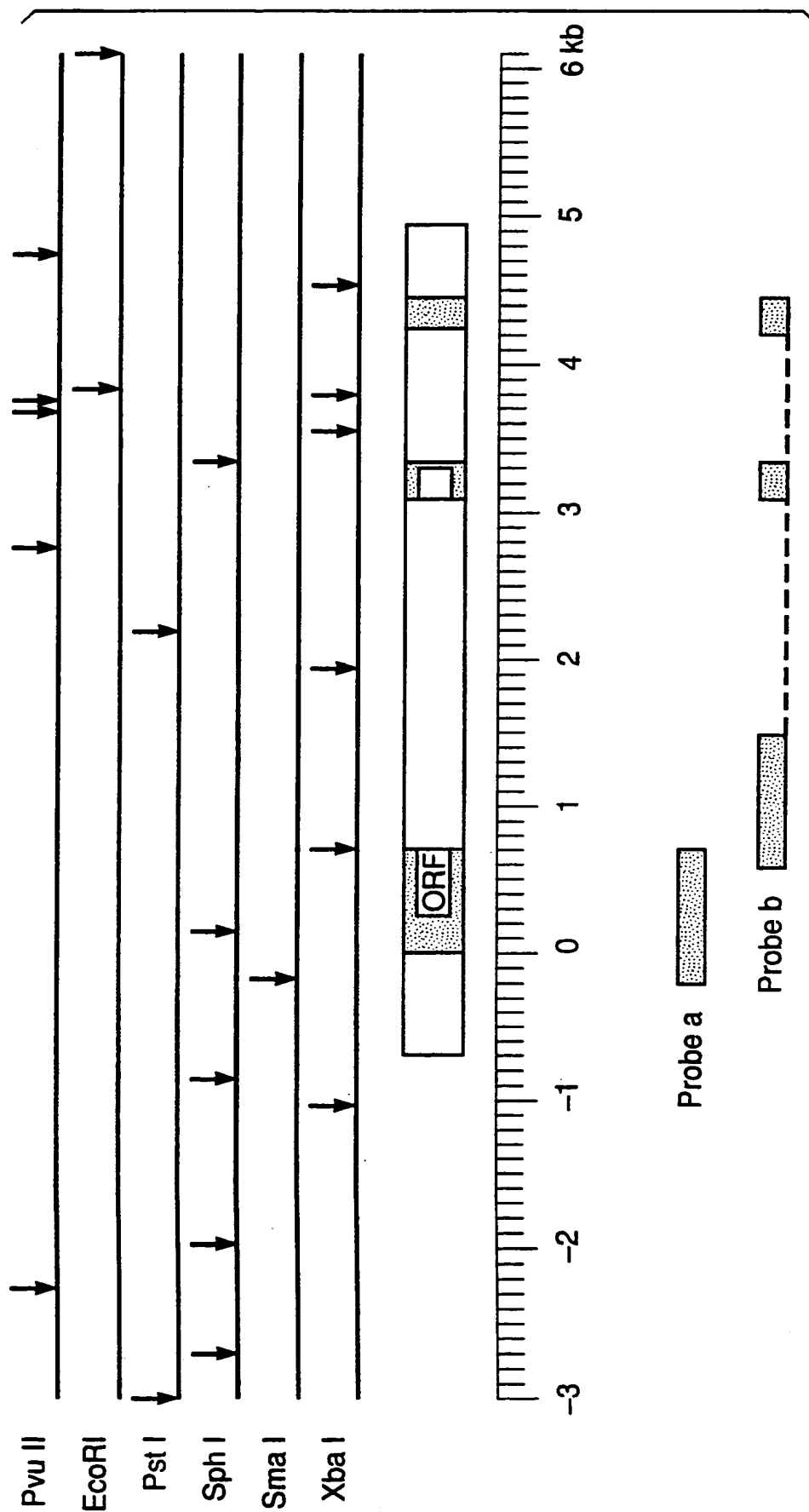
**FIG. 3**

5/13

**FIG. 4**

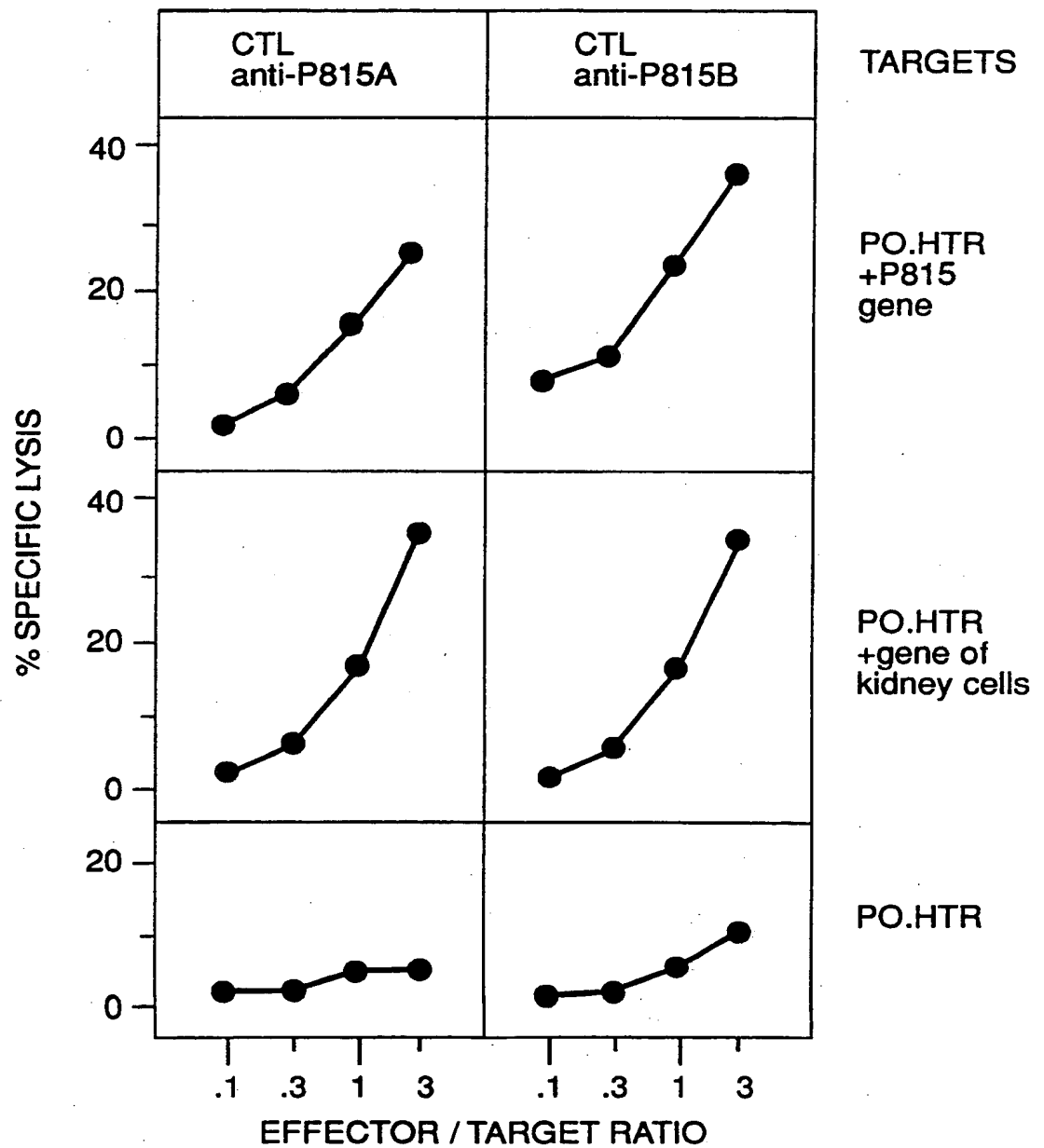
6/13

FIG. 5

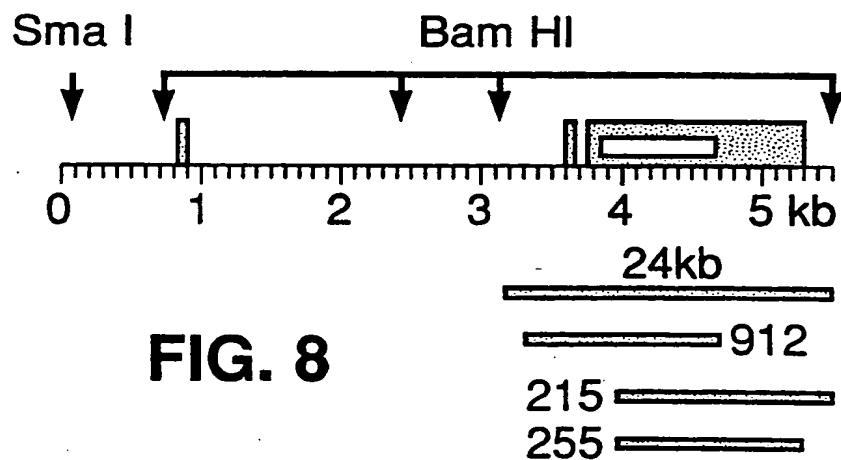
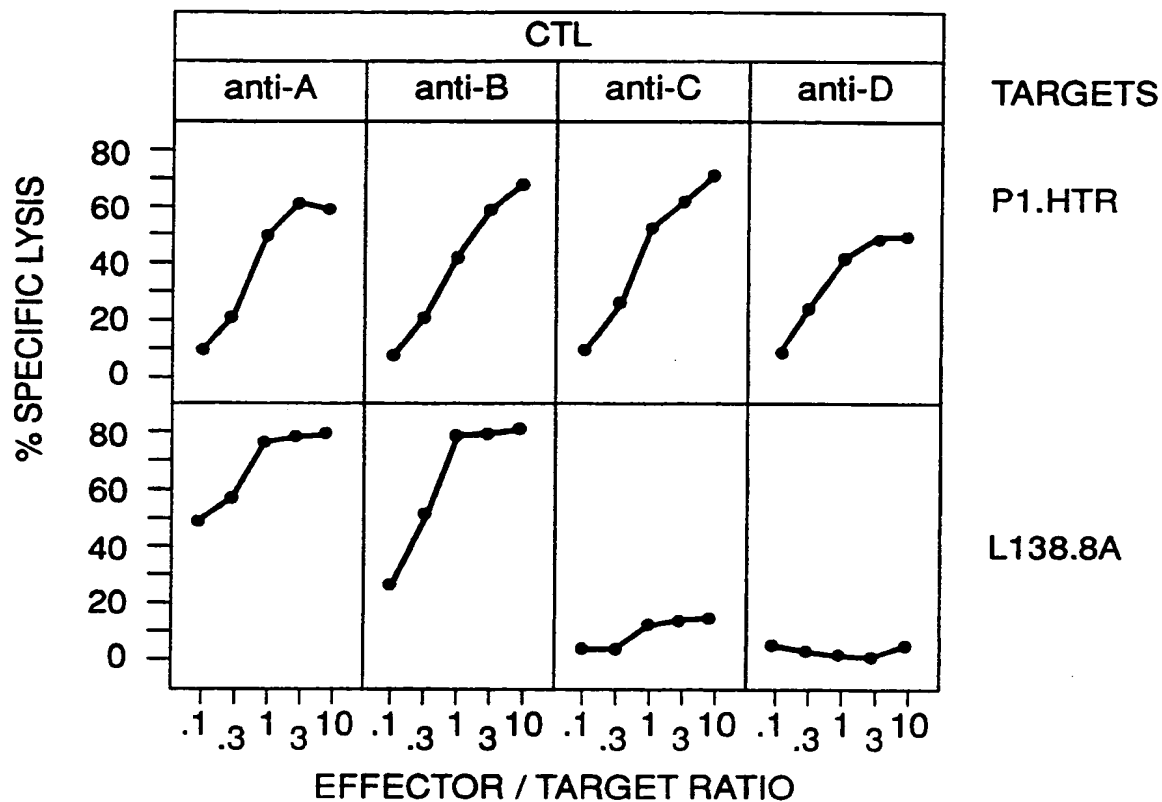




7/13

**FIG. 6**

8/13

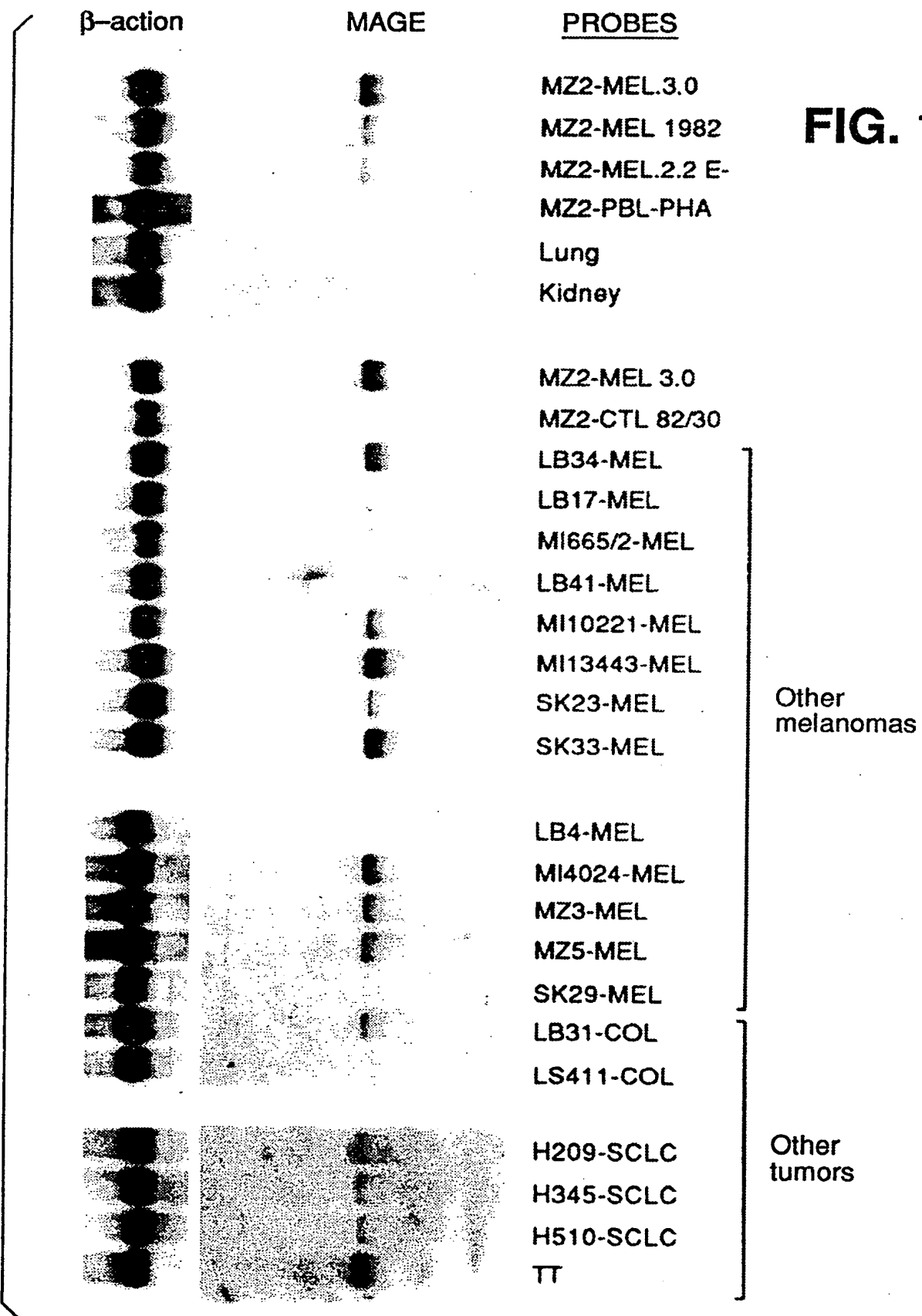
**FIG. 7****FIG. 8**

## FIG. 9

MAGE-3 III CCTCCCCAGAGTCCTCAGGGAGCCTCCagCCtCCCCACTACCATgAACTaCCtCtctgGAGcCAAtCCTaTGAGGacTCCAGCAaCCaaGAAGAGGAGG  
 MAGE-2 II CCTCCCCaAGTCCTCAGGGAGCCTCCagCTTctCgACTACCATCAACTaCtACTcttctgGAGaCAAtCCgATGAGGGcTCCAGCAaCCaaGAAGAGGAGG  
 MAGE-1 I CCTCCCCaGAGTCCTCAGGGAGCCTCCGCCCTTtCCCACTACCAtCAACtTCACtCGACAGAGGCAACCCAGtGAGGGtTCCAGcAGCCGtGAAGAGGAGG  
 225 CHO-8  
 III GGCCAAAGCACCTtcccTgACC-TGGAGTCCgaGTTCCaAGCAGcAcTCAGTAgGAAGGTGGCCcGAgTTGGTTcaTTTTCTGTCTCCTCAAgTATCGAGGCCA  
 II GGCCAAAGaAtgTtTcccgaCCtTGGAGTCCGAGTtCCaAGCAGcAAATCAGTAgGAAGATGGtTGAGTTGGTTcaTTTTCTGTCTCCTCAAgTATCGAGGCCA  
 I GGCCAAAGcACCTtCTtGTATCC-TGGAGTCCtTGTtCCGAGcAGTAATcACTaAGAAAGGTGGCTGAItTGGTTGGTTtCTGTCTCCTCAAAATATCGAGGCCA  
 325  
 III GGGAGCCgGTCACAAAGGCAGAAATGCTGGgGAGTGTCTgTCgGAAATTgCAGtAtTtctTTCTGTgATCTTCaGCAAAAGCTTccagTTCCTTGCAGCT  
 II GGGAGCCgGTCACAAAGGCAGAAATGCTGGAGAGTGTCTCTCAGAAATTgCCAGgACTtctTTTCCcGtGATCTTCaGCAAAAGCCTCCcGAGTaCTTGCAGCT  
 I GGGAGCCAGTCACAAAGGCAGAAATGCTGGAGAGTGTCTATCAAAATATACAAAGcACtGTtTTTCTCTGAGATCTTCCGGCAAAAGCCTCTcGAGtTCCtTGCAGCT  
 425 SEQ-4  
 III GGTCTTTTGGCATcGAgcTGATGGAAgTgGACCCCAcCGGCCACTtGTAcATctTTGcCACCtGGCCtGggCcCTCTCTCTAcGATGGCCCTGCTGGGTGAcAAAT  
 II GGTCTTTTGGCATcGAgGTGgtGGAAgTgGtCCCCATcAgCCCACTtGTAcATcCTTGTcACCTGGCCtGggCcCTCTCTCTAcGATGGCCCTGCTGGGGcGAcAAAT  
 I GGTCTTTTGGCATtGACGTGAAGGAAGCAGACCCcCAGcAGTGTCTGTcACCTGGcTGTCTCTCTATGATGGCCCTGCTGGGTGATAT.  
 525  
 III CAGATCATGCCCAAGgCAGGCCtCCTGTATAATcGTCTTGGCCATaATcGCAAgAGAGGGCGAcTgTGCcCCTGAGGAGaAAATCTGGGAGGAGCTGAGTG  
 II CAGgTCATGCCCAAGACAGGCCcTCCTGTATAATcGTCTTGGCCATaATcGCAATaGAGGGCGAcTgTGCcCCTGAGGAGaAAATCTGGGAGGAGCTGAGTa  
 I CAGATCATGCCCAAGACAGGCCtTCTGTATAATtGTCTTGGTcATGATGCAATGGAGGGCGCCATGCTCTGAGGAGGAAATCTGGGAGGAGCTGAGTG  
 625 CHO-9

9/13

10/13



11/13

**FIG. 11**Expression of  
antigen MZ2-E  
after transaction\*\*

		EXPRESSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:		
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§	
Cells of patient MZ2	melanoma cell line MZ2-MEL3.0	+	++++	++++	++++	+	+	
	tumor sample MZ2 (1982)	+	+++	+++	+++			
	antigen-loss variant MZ2-MEL2.2	+	-	+++	+++	-	-	
	CTL clone MZ2-CTL82/30	-	-	-	-			
	PHA-activated blood lymphocytes	-	-	-	-			
Normal tissues	Liver	-	-	-	-			
	Muscle	-	-	-	-			
	Skin	-	-	-	-			
	Lung	-	-	-	-			
	Brain	-	-	-	-			
	Kidney	-	-	-	-			
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	++++	++++	+	+-	
	MI6652-MEL	-	-	-	-	-	-	+
	MI10221-MEL	+	-	++	+++	-	-	+
	MI13443-MEL	+	+++	++++	++++	+	+	
	SK33-MEL	+	-	++++	++++	-	-	-
	SK23-MEL	+	-	++++	++++	-	-	+
Melanoma cell lines of other patients	LB17-MEL	+	+	++++	++++	-	-	-
	LB33-MEL	+	-	+++	+++	-	-	-
	LB4-MEL	-	-	-	-	-	-	
	LB41-MEL	-	-	-	-	-	-	
	MI4024-MEL	+	+++	++++	++++	-	-	
	SK29-MEL	-	-	-	-	-	-	
	MZ3-MEL	+	+	++++	++++	-	-	
	MZ5-MEL	+	-	++++	++++	-	-	
Melanoma tumor sample	BB5-MEL	+	+++	++	+++			
Other tumor cell lines	small cell lung cancer H209	+	-	++++	++++			
	small cell lung cancer H345	+	-	++++	++++			
	small cell lung cancer H510	+	-	++++	++++			
	small cell lung cancer LB11	+	+	++++	++++			
	bronchial squamous cell carcinoma LB37	+	-	-	+++			
	thyroid medullary carcinoma TT	+	++++	+++	++++			
	colon carcinoma LB31	+	-	+++	++++	-		
	colon carcinoma LS411	-	-	-	-			
Other tumor samples	chronic myeloid leukemia LLC5	-	-	-	-			
	acute myeloid leukemia TA	-	-	-	-			

\* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

\*\* Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

12/13

# FIG. 12

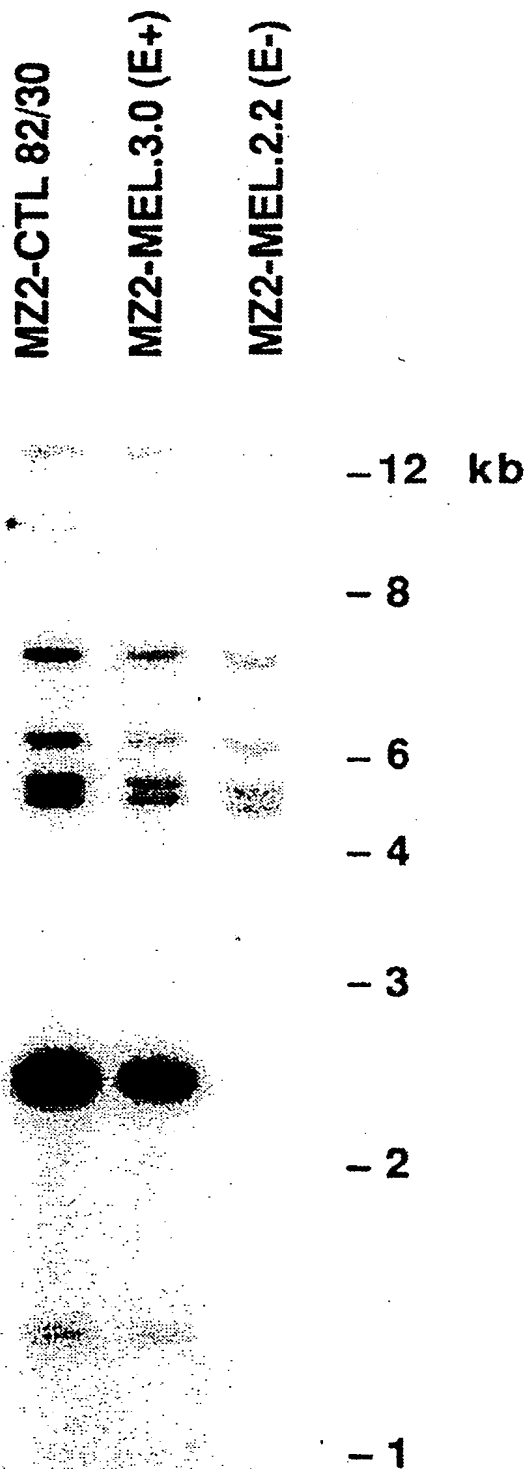
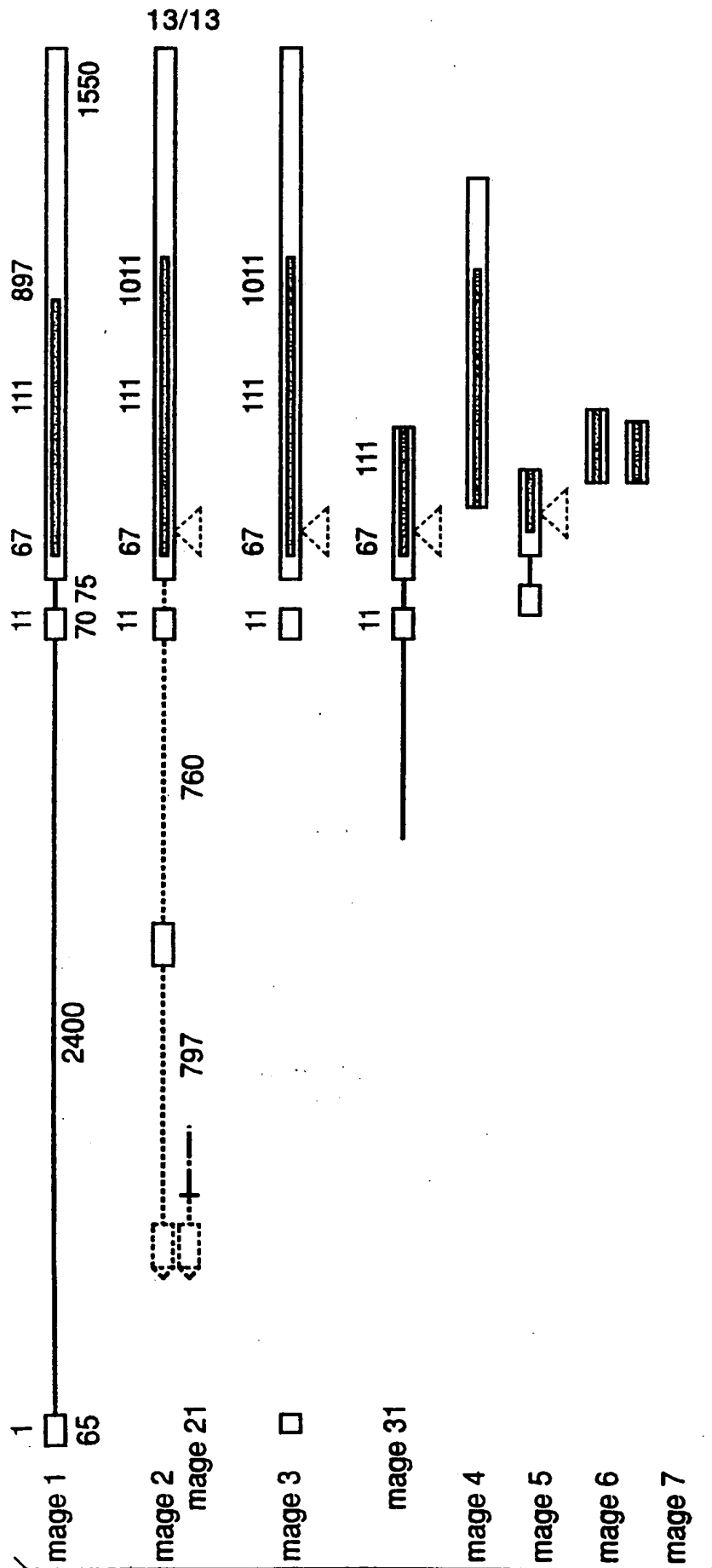


FIG. 13



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/04354

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/25; 530/350, 387; 424/88, 450; 435/320.1, 7.2, 7.1, 243, 252.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X</u> Y	Journal of Experimental medicine, Volume 172, issued July 1990, Sibille et al, "Structure of the Gene of tum- Transplantation Antigen P198: A Point Mutation Generates a New Antigenic Peptide", pages 35-45, see entire document.	<u>1-63</u> 121-134
Y	International Journal of Cancer, Volume 30, issued 1982, Liao et al, "Human Melanoma-Specific Oncofetal Antigen Defined By A Mouse Monoclonal Antibody", pages 573-580, see entire article.	121-133
X	Journal of the National Cancer Institute, Volume 72, No. 1, issued January 1984, Gupta et al., "Studies of a Melanoma Tumor-Associated Antigen Detected in the Spent Culture Medium of a Human Melanoma Cell Line by Allogenic Antibody. II. Immunobiologic Characterization", pages 75-82, see entire article.	154, 155
X	Journal of Experimental Medicine, Volume 152, issued November 1980, Boon, et al., "Immunogenic Variants Obtained by Mutagenesis of Mouse Mastocytoma P815 II. T Lymphocyte Mediated Cytolysis", pages 1184-1193, see entire article.	64-76, 152, 153



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 SEPTEMBER 1992

Date of mailing of the international search report

15 SEP 1992

Name and mailing address of the ISA/  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

LYNETTE F. SMITH

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*



## INTERNATIONAL SEARCH REPORT

International application No.

US92/04354

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cell, Volume 58, issued 28 July 1989, Lurquin et al, "Structure of the Gene of Tum-Transplantation antigen P91A: The Mutated Exon Encodes a Peptide Recognized with L <sup>d</sup> by Cytolytic T Cells", pages 293-303, see entire article.	1-63, 165-172
Y,E	US, A, 5,141,742 (Brown et al) 25 August 1992, columns 5-9.	77-100, 135-144, 156-164
Y	Journal of Virology, Volume 49, No. 3, issued March 1984, Mackett, et al., "General Method for Production and Selection of Infectious Vaccinia Virus Recombinants Expressing Foreign Genes", pages 857-864, see entire document.	47-63
Y	Cancer Research, Volume 48, issued 01 June 1988, Fearon, et al, "Induction in a Murine Tumor of Immunogenic Tumor Variants by Transfection with a Foreign Gene", pages 2975-2980, see entire article.	77-100
Y	Cancer Research, Volume 39, issued May 1979, Gupta et al, "Isolation and Immunochemical Characterization of Antibodies from the Sera of Cancer Patients Which are Reactive against Human Melanoma Cell Membranes by Affinity Chromatography", pages 1683-1695, see pages 1686-1689.	101-120
Y	Cancer Research, Volume 43, issued July 1983, Morgan et al, "Monoclonal Antibodies to Human Melanoma-associated Antigens: An Amplified Enzyme-linked Immunosorbent Assay for the Detection of Antigen, antibody and Immune Complexes", pages 3155-3159, see entire article.	101-120
Y	Journal of Surgical Research, Volume 48, issued 1990, Wong et al, "Immunochemical Characterization of a Tumor-Associated Antigen Defined by a Monoclonal Antibody", pages 539-546, see entire article.	101-120

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/04354

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61K 35/14, 39/00, 37/22; C07K 3/00, 13/00, 15/00, 17/00; C12Q 1/68, 1/00, 15/00; C12N 1/20, 1/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/25; 530/350, 387; 424/88, 450; 435/320.1, 7.2, 7.1, 243, 252.32